

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
6 June 2002 (06.06.2002)

PCT

(10) International Publication Number
WO 02/43775 A2

(51) International Patent Classification⁷: A61K 49/00

(21) International Application Number: PCT/US01/46151

(22) International Filing Date:
20 November 2001 (20.11.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/252,269 20 November 2000 (20.11.2000) US

(71) Applicant: BOARD OF REGENTS UNIVERSITY OF TEXAS SYSTEM [US/US]; Gpdfrey, Cullen, M., University of Texas System, 201 W. 7th Street, Austin, TX 78757 (US).

(72) Inventors: SHERRY, A., Dean; 1634 Spanky Branch Drive, Dallas, TX 75248 (US). ZHANG, Shanrong; 5200 Meadowcreek Drive, #1058, Dallas, TX 75248 (US). WU, Kuangcong; 2524 Cima Hill Drive, Plano, TX 75023 (US).

(74) Agent: GAINES, Charles, W.; Hitt Gaines & Boisbrun, PC, P.O. Box 832570, Richardson, TX 75083 (US).

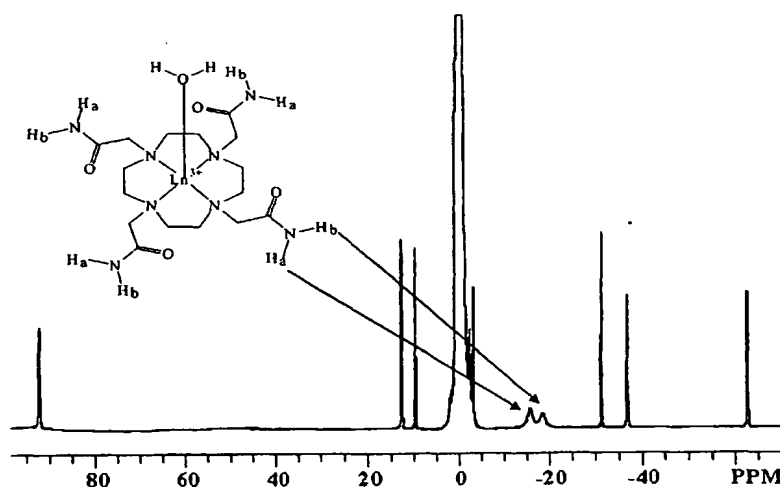
(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— without international search report and to be republished upon receipt of that report

[Continued on next page]

(54) Title: PARAMAGNETIC METAL ION-BASED MACROCYCLIC MAGNETIZATION TRANSFER CONTRAST AGENTS AND METHOD OF USE



(57) Abstract: The present invention is directed, in general, to contrast agents (CA), and methods and systems of using such agents for producing image contrast based on a magnetization transfer (MT) mechanism. The CA comprises a tetraazacyclododecane ligand having pendent arms R, R', R'' and R''' that are amides having a general formula: -CR₁H-CO-NH-CH₂-R₂. R₁ includes organic substituents and R₂ is not hydrogen. A paramagnetic metal ion (M) is coordinated to the ligand. The method, comprises subjecting a CA, in a sample, to a radio frequency pulse. The CA has pendent arms R, R', R'' and R''' comprising organic substituents and the ligand further includes a M and a water molecule. A signal is obtained by applying a radio frequency pulse at a resonance frequency of the water molecule. The magnetic resonance system, comprises a magnetic resonance apparatus and the CA, the agent containing a ligand having the above described general formula.

BEST AVAILABLE COPY



For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

PARAMAGNETIC METAL ION-BASED MACROCYCLIC MAGNETIZATION TRANSFER
CONTRAST AGENTS AND METHOD OF USE

TECHNICAL FIELD OF THE INVENTION

5 The present invention is directed to contrast agents and methods of using contrast agents for altering the magnetic resonance signal of samples, and more particularly, to paramagnetic metal ion-macrocyclic complexes as contrast agents and methods of using such agents for producing image contrast
10 based on a magnetization transfer mechanism.

BACKGROUND OF THE INVENTION

Contrast agents (CAs) are widely used to enhance magnetic
15 resonance imaging (MRI) contrast. The administration of Extrinsic CAs, such as gadolinium (Gd) containing CAs, are thought to achieve contrast by the paramagnetic relaxation effect of a metal-ion to shorten the bulk water relaxation time via rapid exchange of the metal ion's inner-sphere water
20 molecules with bulk solvent. The ability to turn CAs on or off raises the possibility of using such CAs to measure changes in physiological status of tissue samples. For example some CAs exclude Gd from the inner sphere while inactive, and then on activation expose bulk water to a rapidly exchanging water
25 site on the Gd. However, the utility of such CAs in living subjects may be limited by toxicity and undesirable spin-spin lattice relaxation time (T_2^*) effects. In addition, CAs having a slow rate of water exchange are disfavored because this hampers the metal-ion's ability to shorten the bulk water
30 relaxation time and thus enhance contrast.

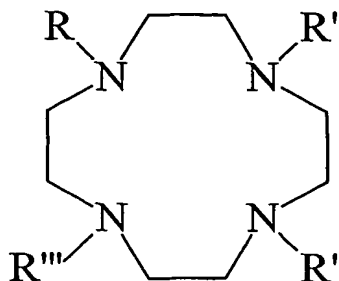
Chemical exchange saturation transfer (CEST) is an alternative technique to enhance MRI contrast. Contrary to the above described CAs, CEST favors CAs having a slow rate of water exchange. For example, intrinsic metabolites with slowly exchangeable NH or OH sites may be saturated to produce a direct intensity decrease in the bulk water signal by Magnetization Transfer (MT). It may also be possible to develop MT-based CA that turn on an off in response to a physiologic parameter, such as pH, if the exchange rate of the NH or OH sites are sensitive to changes in that parameter. However, because the chemical shifts of such diamagnetic NH and OH groups in intrinsic metabolites are typically within 5 ppm of bulk water, it can be difficult to avoid off-resonance saturation of bulk water or tightly protein-bound water in tissue samples. Extrinsic CAs may similarly enhance image contrast by MT. Such CAs, however, rely on the chemical exchange of NH or OH functional groups covalently bonded to the CA and close to the resonance frequency of bulk water.

Accordingly, what is needed is an improved MT-based CA that is amenable to discriminating and reporting on the changing metabolic status of a target sample in contrast to its surroundings.

SUMMARY OF THE INVENTION

25

To address the deficiencies of the prior art, the present invention, in one embodiment, provides a contrast agent comprising: a tetraazacyclododecane ligand having a general formula as follows:



wherein pendent arms R, R', R'' and R''' are amides having a general formula: $-CR_1H-CO-NH-CH_2-R_2$, wherein R_1 includes organic substituents and R_2 is not hydrogen; and a
 5 paramagnetic metal ion coordinated to the tetraazacyclododecane ligand.

In another embodiment, the present invention provides a method of using a magnetic resonance (MR) contrast agent, comprising: subjecting a contrast agent contained within a
 10 sample to a radio frequency pulse wherein the contrast agent is a tetraazacyclododecane ligand having the above described general formula, wherein pendent arms R, R', R'' and R''' comprise organic substituents and the tetraazacyclododecane ligand further includes a paramagnetic metal ion coordinated
 15 to the tetraazacyclododecane ligand and a water molecule associated with the tetraazacyclododecane ligand; and obtaining a magnetization transfer signal by applying a radio frequency pulse at a resonance frequency of the water molecule.

Yet another embodiment provides a magnetic resonance
 20 system, comprising: a magnetic resonance (MR) contrast agent, wherein the MR agent tetraazacyclododecane ligand has the above described general formula, wherein pendent arms R, R', R'' and R''' comprise organic substituents and the
 25 tetraazacyclododecane ligand further includes a paramagnetic

metal ion coordinated to the tetraazacyclododecane ligand and a water molecule associated with the tetraazacyclododecane ligand, wherein the MR contrast agent produces a magnetization transfer signal when subjected to a radio frequency pulse; and
5 a magnetic resonance apparatus.

The foregoing has outlined, preferred and alternative features of the present invention so that those skilled in the art may better understand the detailed description of the invention that follows. Additional features of the invention
10 will be described hereinafter that form the subject of the claims of the invention. Those skilled in the art should appreciate that they can readily use the disclosed conception and specific embodiment as a basis for designing or modifying other structures for carrying out the same purposes of the
15 present invention. Those skilled in the art should also realize that such equivalent constructions do not depart from the spirit and scope of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

20

For a more complete understanding of the invention, reference is now made to the following descriptions taken in conjunction with the accompanying drawing, in which:

FIGURE 1 illustrates a method of using a magnetic
25 resonance contrast agent according to the present invention;

FIGURE 2 illustrates a magnetic resonance system according to the present invention;

FIGURE 3 illustrates the dependence of τ_M^{298} on the radius of the central lanthanide ion for a series of lanthanide ion
30 (Ln^{3+}) complexes of the present invention;

FIGURE 4 illustrates an exemplary ^1H NMR spectrum of the $\text{Eu}(\text{L})^{3+}$ complex produced according to the present invention in the absence of a saturating pulse, and a MT profile for the complex;

5 FIGURE 5 illustrates an exemplary ^1H NMR spectrum of the $\text{Pr}(\text{L})^{3+}$ complex produced according to the present invention in the absence of a saturating pulse, and a MT profile for the complex;

10 FIGURE 6 illustrates an exemplary ^1H NMR spectrum of the $\text{Nd}(\text{L})^{3+}$ complex produced according to the present invention in the absence of a saturating pulse, and a MT profile for the complex;

15 FIGURE 7 illustrates an exemplary ^1H NMR spectrum of the $\text{Yb}(\text{L})^{3+}$ complex produced according to the present invention in the absence of a saturating pulse, and a MT profile for the complex;

20 FIGURE 8 illustrates exemplary MR images of a sample contained the $\text{Eu}(\text{L})^{3+}$ complex (inner cylinder) produced according to the present invention in the absence and presence of a saturating pulse at $\pm\Delta\omega$ for bound water, and corresponding difference images;

25 FIGURE 9 illustrates exemplary MR images of a sample contained the $\text{Nd}(\text{L})^{3+}$ complex (inner cylinder) produced according to the present invention in the absence and presence of a saturating pulse at $\pm\Delta\omega$ for bound water, and corresponding difference images;

FIGURE 10 illustrates an exemplary relationship of the MT effect versus saturation duration time for the $\text{Eu}(\text{L})^{3+}$ complex;

30 FIGURE 11 illustrates an exemplary relationship of the MT effect versus saturation power for the $\text{Eu}(\text{L})^{3+}$ complex;

FIGURE 12 illustrates exemplary MR images of a sample containing the Nd(1)^{3+} complex (inner cylinder) produced according to the present invention in the absence and presence of a saturating pulse at $\pm\Delta\omega$ for bound water with different saturation powers, and corresponding difference images;

FIGURE 13 illustrates an exemplary ^1H NMR spectrum of the Eu(2)^- complex produced according to the present invention in the absence of a saturating pulse;

FIGURE 14 illustrates an exemplary relationship the pH dependence of the τ_M^{298} for the bound water molecule of the Eu(2)^- complex of the present invention;

FIGURE 15 illustrates an exemplary relationship the pH dependence of the τ_M^{298} for protons associated with the amides in the pendent arms of the Eu(2)^- complex of the present invention;

FIGURE 16 illustrates the pH dependence of the MT effect obtained when saturating the bound water molecule of the Eu(2)^- complex of the present invention;

FIGURE 17 illustrates the relationship between the MT effect obtained when saturating the bound water molecule of the Eu(2)^- and the bound water lifetime, τ_M^{298} , or the exchange limiting regime, $\Delta\omega \cdot \tau_M$;

FIGURE 18 illustrates an exemplary ^1H NMR spectrum of the Eu(2)^- complex produced according to the present invention in the absence of a saturating pulse, and a MT profile for the complex produced at three different levels of saturating power;

FIGURE 19 illustrates exemplary MR images of a sample containing the Eu(2)^- complex (inner cylinder) produced according to the present invention in the absence and presence

of a saturating pulse at $\pm\Delta\omega$ for bound water, and corresponding difference images;

FIGURE 20 illustrates exemplary MR images of a sample containing the $\text{Eu}(2)^+$ complex (inner cylinder) produced according to the present invention in the absence and presence of a saturating pulse at $\pm\Delta\omega$ for amide protons, and corresponding difference images;

FIGURE 21 illustrates exemplary MR images of a sample containing the $\text{Eu}(2)^+$ complex (inner cylinder) produced according to the present invention in the absence and presence of a saturating pulse at $\pm\Delta\omega$ for bound water with different saturation powers, and corresponding difference images;

FIGURE 22 illustrates exemplary MR images of a sample containing the $\text{Eu}(2)^+$ complex (inner cylinder) produced according to the present invention in the presence of a saturating pulse at $\pm\Delta\omega$ for amides protons, and corresponding difference images;

FIGURE 23 illustrates an exemplary ^1H NMR spectrum of the $\text{Yb}(9)^{3+}$ complex produced according to the present invention in the absence of a saturating pulse;

FIGURE 24 illustrates an exemplary ^1H NMR spectra of the $\text{Yb}(9)^{3+}$ complex produced according to the present invention in the absence of a saturating pulse and at different sample pH;

FIGURE 25 illustrates an exemplary relationship of the pH dependence of the τ_M^{298} for protons H_a and H_b associated with the amides in the pendent arms of the $\text{Yb}(9)^{3+}$ complex of the present invention;

FIGURE 26 illustrates an exemplary a series of bulk water ^1H NMR spectra obtained for an aqueous solution of $\text{Yb}(9)^{3+}$ complex of the present invention obtained while applying a

saturating pulse of different duration and centered between the resonance signal of the H_a and H_b associated with the amides in the pendent arms of the complex;

FIGURE 27 illustrates an exemplary relationship between the MT effect obtained for different concentrations of the $Yb(9)^{3+}$ complex of the present invention;

FIGURE 28 illustrates an exemplary pH dependence of the MT effect obtained while applying a saturating radio frequency pulse at one or both of the resonance signal of the H_a and H_b associated with the amides in the pendent arms of the $Yb(9)^{3+}$ complex of the present invention;

FIGURE 29 illustrates an exemplary pH dependence of the MT ratios obtained while applying a saturating radio frequency pulse at one or both of the resonance signal of the H_a and H_b associated with the amides in the pendent arms of the $Yb(9)^{3+}$ complex of the present invention;

FIGURE 30 illustrates exemplary 1H NMR spectra and MR images of a sample containing the $Yb(9)^{3+}$ complex (inner cylinder) produced according to the present invention in the presence of a saturating pulse at $\pm\Delta\omega$ for amides protons, H_a and H_b , associated with the amides in the pendent arms of the complex at two different pHs.

DETAILED DESCRIPTION

It has been found that water exchange rates for water molecules bound to certain paramagnetic metal ion-macrocylic complexes were sufficiently slow that a separate bound water MR signal, substantially up field or downfield (e.g., about ± 6 ppm or more) from the bulk water MR signal, is observable at room temperature in pure water as solvent. Furthermore, this

highly shifted and slowly exchanging bound water molecule may be irradiated to produce magnetization transfer (MT) on bulk water and thereby serve as an effective CA.

The theory of MT had been known for several decades and was widely used in chemistry and biology. See e.g., Forsen S. & Hoffman R.A., 39 J.CHEM.PHYS. 2892 (1963) and 40 J.CHEM.PHYS. 1189 (1964); Dwek, R. A. NUCLEAR MAGNETIC RESONANCE (N.M.R.) IN BIOCHEMISTRY (Oxford University Press, London, 1973); incorporated herein by reference. Theoretically, the extent of observed MT depends on chemical exchange and relaxation:

$$\frac{M_{on}}{M_{off}} = \left(\frac{1}{1 + k_{obs} T_{1sat}} \right) + \left(\frac{k_{obs} T_{1sat}}{1 + k_{obs} T_{1sat}} \right) \exp \left[- \frac{(1 + k_{obs} T_{1sat})}{T_{1sat}} \times t \right] \quad (1)$$

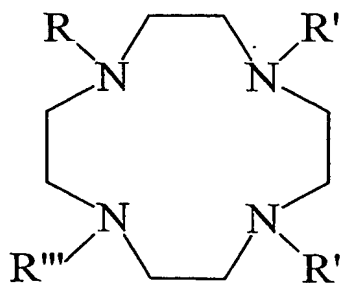
where M_{on} and M_{off} represent the bulk water signal intensity with or without selective Radio Frequency (RF) irradiation at the exchanging sites, respectively. k_{obs} is the pseudo-first order exchange rate between bulk water and the exchanging protons, given by the concentration ratio of the exchanging sites relative to water protons divided by the lifetime of the exchange sites, τ_M . T_{1sat} is the spin-lattice relaxation time of the bulk water protons during saturation of the exchangeable protons. For paramagnetic systems, T_{1sat} is no longer a constant, but is rather described by standard theory of paramagnetic relaxation, summarized in equation (2):

$$\frac{1}{T_{1sat}} = r_1[CA] + \frac{1}{T_{1dia}} \quad (2)$$

Here r_1 is the relaxivity ($\text{mM}^{-1}\text{s}^{-1}$) of the CA, originating from both inner- and outer-sphere paramagnetic contributions. See e.g., Lauffer R.B., 87 CHEM.REV. 901 (1987), incorporated herein by reference.

- 5 To observe a MT effect, the system ideally should be in an exchange limiting regime, defined as $\Delta\omega \cdot \tau_M \geq 1$. The difference in frequency between the MR frequency of the exchanging sites and the MR frequency of bulk water is defined as $\Delta\omega$. The life-time of the exchanging site is defined as τ_M .
- 10 One advantage of certain paramagnetic lanthanide macrocyclic complexes of the present invention displaying a large $\Delta\omega$ is that faster exchange may take place, because τ_M is short, without approaching the exchange limit. Moreover, because the resonance frequency of the exchangeable water molecule site is
- 15 distant from bulk water. For example, $\Delta\omega$ corresponds to about ± 6 ppm to about ± 500 ppm, and preferably about ± 16 ppm to ± 500 ppm. It is therefore possible to saturate the exchanging site while minimizing off-resonance saturation (i.e., direct saturation) of bulk water, and resulting non-specific,
- 20 detrimental decreased MR signal intensity.

The CA of the present invention includes a tetraazacyclododecane ligand having a general formula as follows:



25

The pendent arms R, R', R'' and R''' are amides having a general formula: $-CR_1H-CO-NH-CH_2-R_2$, R_1 includes organic substituents and R_2 is not hydrogen.

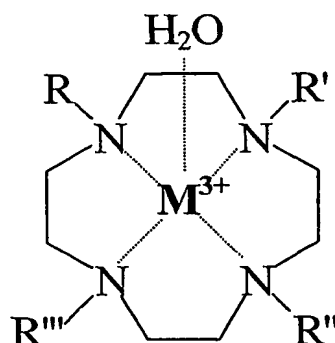
The CA may further include a water molecule, referred to
5 as a bound water molecule, as the exchanging group. The bound water molecule is associated with the tetraazacyclododecane ligand and paramagnetic metal ion such that the bound water molecule has a $\Delta\omega \cdot \tau_M \geq 1$. In certain advantageous embodiments of the CA, the bound water has a $\Delta\omega \geq 6$ ppm. In other
10 embodiments of the CA, the bound water molecule has the $\tau_M \geq 1$ μs .

The CA further comprises a paramagnetic metal ion coordinated to the tetraazacyclododecane ligand. Any paramagnetic metal ion is within the scope of the invention,
15 although certain metal ions of the lanthanide group are preferred. As further detailed in the Experiments below, at magnetic field strengths below 4.7 Tesla (T) the preferred metal ion includes one of Eu^{3+} , Tb^{3+} , Dy^{3+} or Ho^{3+} . At higher field strengths the metal ion may also include Pr^{3+} , Nd^{3+} , Sm^{3+} ,
20 Er^{3+} or Tm^{3+} .

In certain preferred embodiments of this CA, R_2 does not have a proton exchangeable group and is not hydrogen. In other preferred embodiments, R_2 may comprise alkyl groups having 20 carbon atoms or less, cycloalkyl groups having 20
25 carbon atoms or less, alkyloxy groups having 20 carbon atoms or less, alkyl ethers having 10 oxygen atoms or less and 20 carbon atoms or less, or polyols having 20 carbon atoms or less. R_1 may comprise H, alkyl groups having 20 carbon atoms or less, cycloalkyl groups having 20 carbon atoms or less,
30 alkyloxy groups having 20 carbon atoms or less, alkyl ethers

having 10 oxygen atoms or less and 20 carbon atoms or less, or polyols having 20 carbon atoms or less.

Another embodiment of the present invention is a method 100 of using a magnetic resonance (MR) contrast agent. As illustrated in FIGURE 1, the method comprises subjecting 110 a contrast agent contained within a sample to a radio frequency (RF) pulse. Here, the contrast agent (CA) is a tetraazacyclododecane ligand having the general formula as presented below:



10

where pendent arms R, R', R'' and R''' comprise organic substituents and the tetraazacyclododecane ligand further includes a paramagnetic metal ion (M^{3+}) coordinated to the tetraazacyclododecane ligand and a water molecule (bound H_2O) associated with the tetraazacyclododecane ligand. The method 100 further comprises obtaining 120 a magnetization transfer (MT) signal by applying 130 a radio frequency pulse at a resonance frequency of the water molecule. In a preferred embodiment of method 100 the water molecule, referred to as a bound water molecule, has a $\Delta\omega \cdot \tau_M \geq 1$. Optionally, method 100 may further include producing a magnetization transfer MR image 140 from the magnetization transfer signal. Method 100 may optionally further include applying a saturating pulse radio 150 frequency pulse to produce the magnetization

transfer signal. Those skilled in the art, however, understand that other means of producing magnetization transfer, for example applying a frequency specific 180° pulse or multi-dimensional NMR techniques, are within the scope of the present invention.

In one embodiment of method 100 the CA has at least one, and preferably four, pendent arms containing an amide group. Such embodiments of method 100 include obtaining the magnetization transfer signal 120 by applying a radio frequency pulse 160 at a resonance frequency of the protons associated with the amide. As further demonstrated in the Experiments below, the radio frequency pulse may be applied at the resonance frequency of one or all of the exchangeable protons associated with the amide to produce a magnetization transfer signal that is sensitive to pH. The relationship between pH and the magnetization signal may be further be preferably expressed as a ratio of the MT signal obtained while applying the radio frequency pulse one exchangeable amide proton relative to the MT signal obtained while applying the radio frequency pulse to a second or all of the exchangeable amide protons.

In certain preferred embodiments of method 100, where the pendent arms of the CA each contain an amide group, the pendent arms are identical and have the general formula:

$-\text{CHR}_1-\text{CO}-\text{NR}_2-\text{R}_3$, wherein R_1 , R_2 and R_3 comprise organic substituents. In one preferred embodiment of this type of CA, the R_1 and R_2 are H, and the R_3 has the general formula: $-(\text{CH}_2)_n\text{COOR}_4$ where $n = 1-20$, and the R_4 is H, a Group IA or IIA metal ions or alkyl group containing from one to twenty carbon atoms. In these preferred embodiments, the paramagnetic metal ion is preferably Tb^{3+} , Dy^{3+} or Ho^{3+} at magnetic field strengths

less than 4.7 T, or additionally, Eu^{3+} , Pr^{3+} or Nd^{3+} at higher magnetic field strengths (i.e., 4.7 to 11.75 T). In a second preferred embodiment of the above-described CA, the R_1 and R_2 are H, and the R_3 has the general formula: $-(\text{CH}_2)_n\text{P}(\text{O})(\text{OR}_4\text{OR}_5)$ where $n = 1-20$; R_4 is H, an alkaline earth metal ion of Groups IA or IIA or an alkyl group containing one to twenty carbon atoms; and R_5 also is H, an alkaline earth metal ion of Groups IA or IIA or an alkyl group containing one to twenty carbon atoms. In a third preferred embodiment of the above-described CA, the R_1 and R_2 may be H, and the R_3 has the general formula: $-(\text{CH}_2)_n\text{R}_4$ where $n = 1-20$; and R_4 is Pyridine (Py) or Phenol (Ph).

In another embodiment of method 100, the CA has pendent arms R and R'' that are identical, the pendent arms R' and R''' are identical, and the pendent arms R' and R''' are not equal to the pendent arms R and R'' . In one preferred embodiment of the above-described CA, the pendent arms R and R'' have the general formula: $-\text{CHR}_1-\text{CO}-\text{NH}-\text{R}_2$; and the pendent arms R' and R''' have the general formula: $-\text{CHR}_3-\text{CO}-\text{NH}-\text{R}_4$ where R_1 , R_2 , R_3 , and R_4 comprise organic substituents, and the R_2 is not equal to the R_4 . Yet another embodiment of the present invention is a magnetic resonance system 200. As illustrated in FIGURE 2, the system 200 comprises a magnetic resonance (MR) contrast agent (CA) 210, wherein the MR agent contains a tetraazacyclododecane ligand having the same general formula described for method 100. The CA 210 includes pendent arms R, R' , R'' and R''' that comprise organic substituents. CA 210 further includes a paramagnetic metal ion coordinated to a water molecule, referred to as a bound water molecule, associated with the tetraazacyclododecane ligand, where the MR

contrast agent produces a magnetization transfer signal when subjected to a radio frequency pulse. In certain embodiments of the present invention, the CA includes at least one and up to twenty tetraazacyclododecane ligands. Such ligands may be
5 covalently or noncovalently bonded to a carrier, such as a protein or polymer, comprising a portion of the CA. Collecting several such ligands, and associated metal ions and bound water molecules, allows effective MT contrast to be achieved at lower concentrations of CA. The system 200 further
10 comprises a magnetic resonance apparatus 220. One of ordinary skill in the art understands that the MR apparatus may include all the hardware and software components necessary to produce magnetic resonance spectra or images.

The system 200 may further comprise a sample 230 that
15 contains the CA 220 within it. The sample includes living subject including animal, for example human, species, or a portion of fluid or tissue withdrawn from the subject. Alternatively, the sample 230 containing the CA 220 may be an inanimate object, or contain other non-living material. In
20 one preferred embodiment of the MR system 200, the magnetic resonance apparatus 210 produces a magnetization transfer image 240 of the sample 230 from the magnetization transfer signal. Such a system 200 may preferably produce the image by applying the radio frequency pulse at a resonance frequency of
25 the bound water molecule 250. Alternatively the radio frequency signal may be applied at the resonance frequency of protons associated with an amide included in one or more of the pendent arms of the CA 260.

In certain preferred embodiments of the MR system 200,
30 the magnetic resonance apparatus produces a magnetization

transfer difference signal 255 by applying the radio frequency pulse at a $\Delta\omega$ of the bound water molecule, acquiring the magnetization transfer signal and subtracting the signal from a MR signal obtained by applying a radio frequency pulse at -
5 $\Delta\omega$. A difference signal may be produced in analogous fashion, by applying the radio frequency pulse at a $\Delta\omega$ of the protons associated with amides 265 in the pendent arms of the CA 210 and subtracting the signal from a MR signal obtained by applying a radio frequency pulse at $-\Delta\omega$. In certain
10 embodiments either difference signals 255, 265 may be further processed by the apparatus 220 to produce a difference image. The magnetic resonance system 200 may further include in the apparatus 220 hardware that produces a saturating pulse 270. The saturating pulse is preferably sufficiently frequency
15 specific to saturate only the exchangeable protons, for example the bound water or the protons associated with the amides contained within the pendent arms of the CA 210. The saturating pulse preferably ranges from about 1 to about 3 seconds.

20 The CA 210 used in the MR system 200 may include any of the embodiments of CA discussed above in the method 100. However, the exchangeable proton within the CA 210, for example bound water, preferably has a $\Delta\omega \cdot \tau_M \geq 1$. In certain
embodiments of the MR system 200, the $\Delta\omega \geq 6$ ppm. In other
25 preferred embodiments of the MR system 200, the $\tau_M \geq 1 \mu s$.

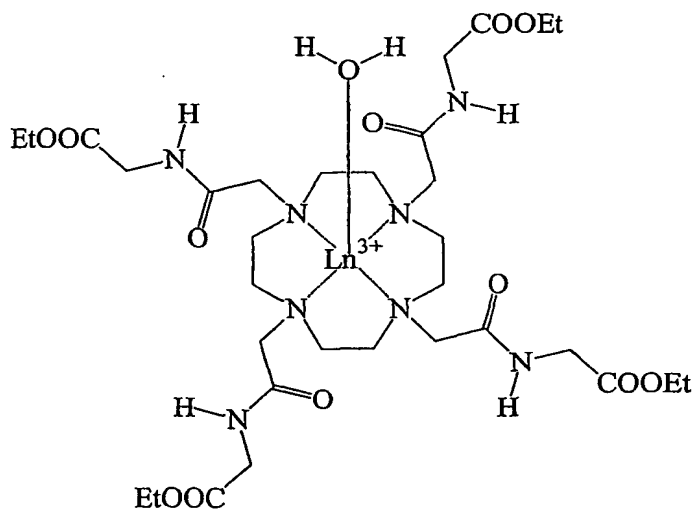
Experiments

Examples of CAs prepared according to the present invention are presented below for illustrative purposes and do not limit the scope of the claimed invention. The synthesis

of polyazamacrocycles having pendent arms comprising organic substitutants has been described in: U.S. Patent 5,428,155, to Sherry A.D. and van Westrenen, J.; Kovacs and Sherry, *pH-Controlled Selective Protection of Polyaza Macrocycles*, 5 SYNTHESIS, 761-63, (July 1997); Zhang S., Winter P., Wu. K. & Sherry A.D., *A Novel Europium(III)-Based Contrast Agent*, 123 J.AM.CHEM.SOC. 1517-18 (2001); Zhang S., Wu. K., Biewer M.C., & Sherry A.D. *¹H and ¹⁷O NMR Detection of a Lanthanide-Bound Water Molecule at Ambient Temperatures in Pure Water*, 40 INORG. 10 CHEM. 4284-90 (2001); which are incorporated herein by reference.

Experiment 1

A first experiment, examined the life times at 298°K, τ_M^{298} , of water molecules bound to various lanthanide-macrocylic 15 complexes of the present invention, and having the general formula, $\text{Ln}(1)^{3+}$, where the four pendent arms R, R', R'' and R''' are all ethyl-acetamidoacetate (i.e., LnDOTA-4AmCE^{3+}), as



depicted below:

20

Macrocylic Complex 1 - LnDOTA-4AmCE^{3+}

As illustrated in FIGURE 3, τ_M^{298} is strongly dependent on the radius of the central lanthanide ion. The plot shows the τ_M^{298} measured for a series of $\text{Ln}(\text{I})^{3+}$ complexes in acetonitrile plus 2-4% water versus the Ln^{3+} ionic radii. Individual τ_M^{298} values were obtained by fitting the temperature dependent ^{17}O NMR bound water line widths according to standard exchange theory. In a separate series of experiments, it was found that τ_M^{298} was about 2-fold shorter when pure water was the solvent. Because the bulk water ^{17}O resonance of $\text{Yb}(\text{I})$ solution was relatively narrow at all temperatures, no attempt was made to determine τ_M^{298} for this complex based on the ^{17}O NMR line width data from bulk water. However, a fit to the bound-water ^1H NMR line width gave a τ_M^{298} of 5.8 μs for $\text{Yb}(\text{I})$, consistent with the trend shown in FIGURE 3. Moreover, ^1H NMR line width fitting gave very similar results for those systems for which the bound water is directly observable.

As further illustrated in Table 1, the proton chemical shifts of bound water in these lanthanide-macrocylic complexes, δ , relative to bulk water, at 0 ppm are generally highly shifted, either more than 6 ppm downfield or upfield (i.e., ± 6 ppm or more), depending upon the properties of the central lanthanide ions. The exchange limit regime, $\Delta\omega \cdot \tau_M$, calculated for different field strengths is also shown in Table 1. At 1.5 Tesla (T), a magnetic field strength presently used by many commercial MRI scanners, the exchange limit regimes, $\Delta\omega \cdot \tau_M$, of $\text{Eu}(\text{I})^{3+}$, $\text{Tb}(\text{I})^{3+}$, $\text{Dy}(\text{I})^{3+}$, and $\text{Ho}(\text{I})^{3+}$ are all greater than 1. At higher fields, however, such as 4.7 T and 11.75 T, more lanthanide complexes have exchange limit regimes greater than 1, with $\Delta\omega \cdot \tau_M$ increasing as field strength

increases. Therefore, favorable MT contrast effects are available for a broad range of lanthanide-macrocylic complexes over a broad range of magnetic field strengths.

TABLE 1

Ln(1) ³⁺ Complexes	Observation of bound water	τ_M (μ s)	δ (ppm)	$\Delta\omega \cdot \tau_M$		
				11.75 T	4.7 T	1.5 T
Pr ³⁺	Yes	20	-60	3.8	1.5	0.5
Nd ³⁺	Yes	80	-32	8.0	3.2	1.0
Sm ³⁺	Yes	320	-4	4.0	1.6	0.5
Eu ³⁺	Yes	382	50	60.0	24.0	7.7
Tb ³⁺	No	31	-600	58.5	23.4	7.5
Dy ³⁺	No	17	-720	38.5	15.4	4.9
Ho ³⁺	No	19	-360	21.5	8.6	2.8
Er ³⁺	No	9	200	5.7	2.3	0.7
Tm ³⁺	Yes	3	500	4.7	1.9	0.6
Yb ³⁺	Yes	3	200	1.9	0.5	0.2

5

Experiment 2

In a second experiment, MT profiles, also known as Z-profiles or CEST profiles, were obtained for lanthanide-macrocylic complexes of the general formula Ln(1)³⁺. FIGURES 4-7 show representative spectra in the absence of saturation (bulk water peak at 0 ppm truncated to make the bound water peaks more visible) and MT profiles for Eu(1)³⁺, Pr(1)³⁺, Nd(1)³⁺ and Yb(1)³⁺ complexes, respectively, all measured at 4.7 T. All experiments were conducted using aqueous 62.5 mM Ln(1)³⁺ adjusted to neutral pH and about 22°C, using an saturation duration time of 1 s, RF power of 16 db, and a 2.5 cm surface coil. FIGURES 4, 5 and 6 illustrate that Eu(1)³⁺, Pr(1)³⁺ and

15

Nd(1)³⁺ all display strong MT properties when a saturating RF pulse is directed at their bound water positions of +50, -45 and -36 ppm, respectively (with bulk water at 0 ppm). Among these three complexes, Eu(1)³⁺, shown in FIGURE 4, had the greatest effect when the saturating pulse was centered at about 50 ppm (arrow): about a 60% decrease in the magnetization of bulk water, M_s , as compared to the magnetization of bulk water in the absence of a saturating pulse, M_o . Importantly, as indicated by a M_s/M_o of about 100%, when the counter-position (i.e., -50 ppm) was saturated, there was no distortion in the bulk water signal. Of course when the saturation pulse is directed to 0 ppm (arrow) there is no signal and therefore M_s/M_o is about 0%. Turning to Yb(1)³⁺, theory suggests that the bound water of Yb(1)³⁺ complex should be at about 200 ppm. Unfortunately, as illustrated in FIGURE 7, no MT effect could be observed for this compound, probably due to its fast exchange, namely an exchange limit regime of less than one ($\Delta\omega \cdot \tau_M = 0.5$).

Experiment 3

A third series of experiments was performed to examine the ability of lanthanide-macrocylic complexes of the general formula Ln(1)³⁺ to enhance MRI contrast by MT. FIGURES 8 and 7(21) demonstrate image contrast obtained using aqueous solutions of 62.5 mM Eu(1)³⁺ and Nd(1)³⁺, respectively. The inner vial contains 62.5 mM Eu(1)³⁺ or Nd(1)³⁺ at neutral pH, while the outer vial is pure water. T₁-weighted spin-echo images (TR/TE = 500 /18 ms, 256x256 data matrix) were obtained at about 22°C and a field strength of 4.7 T. MT was achieved by applying RF irradiation for 1 s, with a power of 16 db by using a 2.5 cm surface coil. FIGURE 8 shows images obtained

with no saturation (left, nosat), saturation at +9800 Hz (middle, Satp) at the resonance frequency of Eu^{3+} -bound water, saturation at -9800 Hz (right, satn), and the corresponding difference images. FIGURE 9 shows analogous images for a phantom with no saturation (left, nosat), saturation at -6400 Hz (middle, Satp) the resonance frequency of Nd^{3+} -bound water, saturation at +6400 (right, satn), and the corresponding difference images. The inner vial contains 62.5 mM Nd(1)^{3+} at neutral pH, while the outer vial is pure water. The irradiation duration time was 2 s, with a power of 41 db by using a 2.5 cm surface coil.

Saturating the bound water at +50 ppm for Eu(1)^{3+} (FIGURE 8) and -32 ppm for Nd(1)^{3+} (FIGURE 9) resulted in MT to bulk water, thereby providing about an 80% decrease in the bulk water signal in the inner vials without disturbing the imaging intensity of the outer vials. These levels of contrast are much better than expected for diamagnetic CAs where the resonance signal of the NH or OH group undergoing chemical exchange is only a few ppm away from the resonance signal of bulk water.

Experiment 4

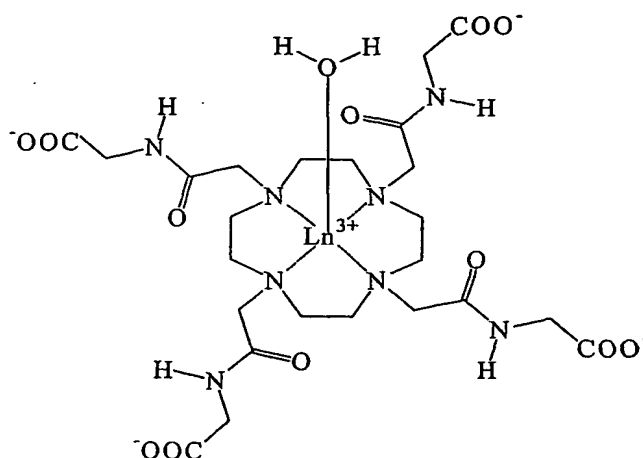
A fourth experiment was performed under similar conditions as described for Experiments 1-3 to investigate the effect of saturation duration time and power on MT to bulk water. FIGURE 10 shows the relationship of the MT effect versus saturation duration time for the Eu(1)^{3+} complex. The theoretical relationship expressed in Equation (1) was fit to this data. The fits reveal that a saturation duration time of one second is sufficient to produce maximum MT effects for the Eu(1)^{3+} complex. Similar analysis of data collected for Pr(1)^{3+}

and Nd(1)^{3+} complexes revealed that a saturation duration time of about 1 to about 2 s was sufficient for these paramagnetic MT-CAs.

FIGURES 11 and 12 show the relationship of MT effects versus saturation power for Eu(1)^{3+} and Nd(1)^{3+} , respectively. Saturation power level is defined as the times the maximum power of 82 db produced by 4.7 T MRI. A saturation duration time of 3 s was used for all experiments conducted on samples containing Eu(1)^{3+} . As illustrated in FIGURE 12, saturation power levels (satpl) were increased to examine the effect of power on MT image contrast obtained using Nd(1)^{3+} . The irradiation duration time was 2 s, and imaging parameters included a $\text{TR/TE} = 500/18$ ms and 64×64 data matrix. As illustrated in FIGURES 11-12, contrast continuously improved with increasing saturation power. However, a practical limit of 30-50 db was found for these CAs given the MRI scanner used in these experiments. Other considerations, such as heating of biological or other heat sensitive samples, due to RF power deposition may also limit the saturation power applied. The CA of the present invention are stable to temperatures of at least 100°C .

Experiment 5

A fifth series of experiments was conducted on a lanthanide-macrocylic complex of the present invention, and having the general formula, Eu(2)^+ , where the four pendent arms R, R', R'' and R''' are all carboxyl-acetamidoacetate,



(i.e., LnDOTA-4AmC⁻) as depicted below:

5

Macrocyclic Complex 2 - LnDOTA-4AmC⁻

FIGURE 13 shows a 500 MHz ¹H NMR spectrum of Eu(2)⁻ complex in an aqueous solution adjusted to pH 7.4 and 25°C. Two sites in the complex (denoted by the symbols *) are chemically exchangeable with bulk water (denoted by the symbol ◇): one is from Eu³⁺-bound water at about 57 ppm, and another is from four equivalent amide protons on the pendent arms at about -6 ppm.

Experiment 6

15 In a sixth series of experiments, the pH dependence of the bound water (FIGURE 14) and the amide (FIGURE 15) protons lifetimes, τ_M^{298} , were determined by variable temperature ¹H NMR line width fitting. The chemically exchangeable bound water and amide protons have different dependence on pH. For bound
20 water protons, the slowest exchange, i.e., largest τ_M^{298} , takes

place at pH 7 (FIGURE 14). Moreover, the pH dependence may be divided into two linear ranges: an acidic range where $\tau_M^{298} = 68.6 \cdot \text{pH} - 172.5$; and a basic range where $\tau_M^{298} = -128.2 \cdot \text{pH} + 1212.6$, respectively. For the amide protons, the slowest exchange is at pH 5.5 (FIGURE 15). In addition, the exchange limiting regimes, $(\Delta\omega \cdot \tau_M)$ calculated from the above data, for the magnetic field strength of 4.7, are all larger than 1, thus indicating that either the bound water or amide protons chemical exchange sites may serve as MT-CAs.

10 Experiment 7

In a seventh experiment, MT profiles were obtained for 62.5 mM aqueous solutions of the $\text{Eu}(2)^+$ lanthanide-macrocylic complex. A saturating RF pulse of 2 s at the resonance frequency of bound water or amide protons, with a power of 41 db, was applied to a 3.5 cm volume coil. FIGURE 16 shows that MT decreases were produced for $\text{Eu}(2)^+$ by saturating bound water proton at about 57 ppm throughout the pH range of 3 to 9. As illustrated in FIGURE 16, the magnitude of MT decrease has a complex dependence upon pH. The same set of data was plotted versus the bound water lifetime, τ_M^{298} , or the exchange limiting regime, $\Delta\omega \cdot \tau_M$, as shown in FIGURE 17. The largest MT occurs for a $\Delta\omega \cdot \tau_M$ ranging from about 12 to about 17, and optimally about 15. FIGURE 18 further demonstrates that the MT effect continues to increase with the magnitude of saturation power applied, up to the maximum power (satpl = 100; corresponding to 82 db) allowed by the MRI instrumentation used in the experiment.

Experiment 8

An eighth series of experiments was performed to examine the ability of the $\text{Eu}(2)^+$ lanthanide-macrocylic complex to

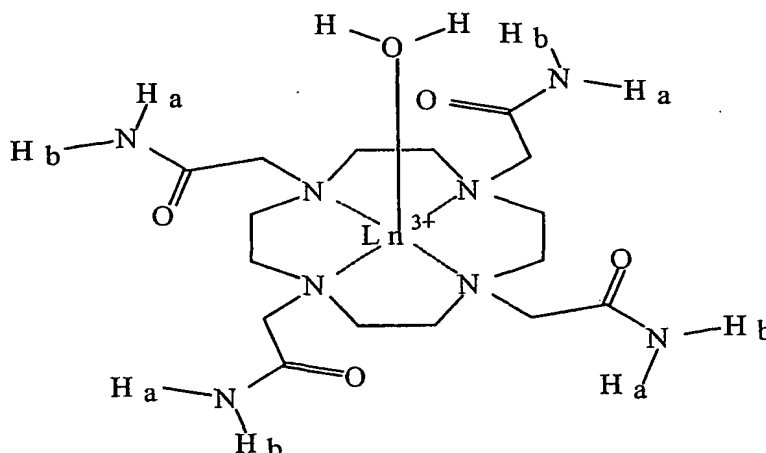
enhance MRI contrast by MT. Images and difference images were collected under conditions similar to that described for Experiment 3, using a sample comprising a inner vial of 62.5 mM $\text{Eu}(2)^+$ and outer vial of water. FIGURES 19 and 20 are MT
5 images obtained while saturating either the bound water or amide protons, respectively. These figures show that the MR signal of either chemically exchangeable protons may be saturated individually to provide large MT contrasts. For saturation of amide protons, however, a lower saturation power
10 was preferred to avoid direct saturation of the bulk water MR signal. Importantly, by virtue of its farther distance from the bulk water MR signal, higher levels of saturating power, and therefore greater MT contrast, may be obtained by saturating the bound water proton MR signal than that obtained
15 by saturating the amide protons. This point is further illustrated in Experiment 9.

Experiment 9

In a ninth series of experiments, analogous to Experiment 4, the effect of saturation power on MT to bulk water using
20 the $\text{Eu}(2)^+$ lanthanide-macrocylic complex was examined. As illustrated in FIGURE 21, a saturating RF pulse was applied at: a) the resonance frequency of bound-water (i.e., $\text{satfreq} = +11500 \text{ Hz}$), and b) an equal frequency away from the bulk water signal but opposite to the bound water signal (i.e., $\text{satfreq} =$
25 -11500 Hz). The difference image (series c) is shown below. As saturation power level (satpl) was increased, image contrast continuously improved. In comparison, when the amide protons of $\text{Eu}(2)^+$ were saturated, as illustrated in FIGURE 22, as power levels were increased there are serious imaging
30 distortions, mostly likely due to direct saturation of the bulk water.

Experiment 10

A tenth experiment was conducted on a lanthanide-macrocylic complex of the present invention, and having the general formula, $\text{Ln}(9)^{3+}$, where the four pendent arms R, R', R'' and R''' are all acetamidoacetate (i.e., LnDOTA-4Am^{3+}), as depicted below:

Macrocylic Complex 9 - LnDOTA-4Am^{3+}

For certain $\text{Ln}(9)^{3+}$ complexes, for example, with Ln equal to Yb or Tm, the bound water molecules are ^1H NMR invisible probably due to the fast exchange between the bound water and the bulk water. However, these complexes are still preferred MT CAs because their ^1H NMR spectra present two sets of amide protons (for example, -14.5 and -17.7 ppm for $\text{Yb}(9)^{3+}$, and -42 and -52 ppm for $\text{Tm}(9)^{3+}$, respectively), which could be saturated to produce MT contrast. FIGURE 23 shows a high resolution ^1H NMR spectrum of an aqueous solution containing $\text{Yb}(9)^{3+}$ adjusted to pH 7.4 and 25°C. Theoretical prediction indicates that a bound water should resonate at about 200 ppm. Unfortunately, the NMR signal was invisible, probably due to its fast exchange with the bulk water. However, the eight

exchangeable amide protons present as two resonance peaks with equivalent intensity, at

-14.5 (H_a) and -17.7 (H_b) ppm; respectively, are visible.

As indicated by their linewidths, illustrated in FIGURE 24, the two exchangeable amide sites have different dependences upon pH. As further illustrated in FIGURE 25, H_b exchanges about 1.3 times faster than that of H_a . The exchange limiting regimes, $\Delta\omega \cdot \tau_M$, of both amide protons at magnetic field strength of 7.05 T are much larger than 1, for a broad range of pH values indicating that $\text{Ln}(9)^{3+}$ complexes in general may be suitable as MT-CAs (TABLE 2). Similar to that discussed above for $\text{Ln}(1)^{3+}$, either too fast or too slow exchange may produce less MT. For the conditions used to obtain the data presented in Table 2, the maximum MT effect appear to be obtained for $\text{Ln}(9)^{3+}$ complexes when the exchange limiting regime is about 15.

TABLE 2

pH	τ_M^{298} , ms		$\Delta\omega \cdot \tau_M^{298}$	
	H_a	H_b	H_a	H_b
6.46	5.00	4.00	139.3	133.7
6.76	2.30	1.78	64.1	59.5
7.06	1.21	0.82	33.6	27.2
7.21	0.92	0.72	25.5	24.2
7.43	0.64	0.52	17.9	17.4
7.84	0.57	0.44	15.9	14.7

Experiment 11

An eleventh series of experiments was conducted to examine the effect of saturation duration on the magnitude of

MT obtained using the Yb(9)^{3+} complex. FIGURE 26 shows a series of bulk water ^1H NMR spectra obtained for an aqueous solution of 5 mM Yb(9)^{3+} adjusted to pH 7.4 and 25°C. The upper series of spectra (a) were obtained while applying a saturating pulse of different duration and centered between the resonance signals of the exchangeable amide protons, with a saturating bandwidth of 1500 Hz. The saturating bandwidth was produced using conventional water elimination technique (i.e., a wetld pulse sequence). See Varian User Manual; Varian NMR, Palo Alto, CA; User Guide, VNMR Version 6.1 software, 1997), incorporated herein by reference. The pulse sequence to produce saturation was modified to include hard loops to shape the pulse train (90° e-burp1, typically repeated from about 0 to about 500 times) with a bandwidth of 1500 Hz (pwwet = 3.0 ms and wetpwr = 28 db). The lower series (b) of spectra, shown inverted to facilitate comparison to series (a), were obtained while applying the same saturating pulse, but on the opposite side and equal distance away from the bulk water MR signal. As the illustrated in FIGURE 26, for a saturating pulse of about 2 s or longer, about a 38% decrease in the bulk water signal was obtained for series (a). In comparison, there was substantially no decrease in the bulk water signal for series (b).

Experiment 12

A twelfth series of experiments was conducted to examine the effect of the Yb(9)^{3+} concentration on the magnitude of MT-based contrast. The concentration dependence of the MT effect obtained for the Yb(9)^{3+} complex, measured at pH 7.4 and room temperature, is further illustrated in FIGURE 27. The curve depicts the best fit of a combination of MT theory and

paramagnetic theory, presented in Equations 1 and 2, to the experimental data. This shows that substantial MT contrast may be obtained at concentrations similar to CA concentrations in clinical use. The results of FIGURE 27 also illustrate that at a certain concentration of CA, the MT effect is independent of concentration. While not restricting the scope of the invention to a particular theory, it is believed that the magnitude of MT effect is strongly linked to the paramagnetic properties of the central metallic ions. That is, although the MT effect should be proportional to concentration at all concentrations, when the concentration gets too high, the spin lattice relaxation time of bulk water (T_{1sat}) becomes too short and dominates the MT effect. For example, for the $Yb(9)^{3+}$ complex, at higher concentrations, paramagnetic relaxation effect due to Yb^{3+} predominates at about 20 mM or higher. Under these conditions, there will be no further MT decrease because the saturated MT signal quickly recovers. As such, an optimal concentration is at about 20 mM of the $Yb(9)^{3+}$ complex. Of course, lower concentration of CA may be preferred for other reasons, such as minimizing the dose of CA necessary to administer to a subject. This experiment also suggests that conventional CAs, using metal ions such as Gd^{3+} for example, will have an even greater effect on shortening T_{1sat} , and therefore are unsuitable for producing MT-based contrast, even at very low concentrations.

Experiment 13

Experiment thirteen was conducted to examine the effect of pH on MT obtained using the lanthanide-macrocylic complexes of the present invention containing two exchangeable sites with different pH responses. Such complexes, for example

Eu(2)⁻ and Yb(9)³⁺, may be used as pH-reporter-CAs in biological applications.

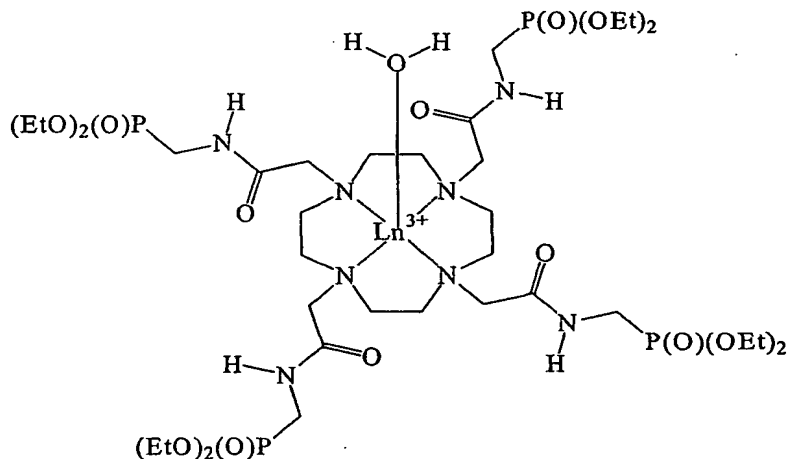
The pH dependence of MT obtained for an aqueous solution containing 30 mM Yb(9)³⁺ at 25°C, is shown in FIGURE 28. The saturating RF pulse was applied at the MR frequency of the amide protons (M_{on}), as described in Experiment 11, using either a bandwidth of 600 Hz when H_a and H_b were saturated individually (pwwet = 7.5 ms and wetpwr = 20 db), or a bandwidth of 1500 Hz when for saturating both amide protons simultaneously. Analogous data was collected with the same type of saturating pulse, but located on the opposite side and equal distance away from the bulk water MR signal (M_{off}). The symbols , Δ and O represent data points for saturating H_a, H_b and both sites, respectively. Similar to that observed for Eu(2)⁻, the relationship between pH and the extent of MT (M_{on}/M_{off}) is complex showing a minimum in MT at about pH 7.4. The same data, expressed as ratios of MT (MTR), are presented in FIGURE 29. MTR is defined as $[1 - M_{\text{off}}/M_{\text{on}}]_{\text{site1}} / [1 - M_{\text{off}}/M_{\text{on}}]_{\text{site2}}$, where site 1 and site 2 refers to saturation applied to amide protons H_a or H_b or both sites, as indicated in the figure legend. To a first approximation MTR, is proportional to $[\tau_M]_{\text{sites2}} / [\tau_M]_{\text{sites1}}$. As illustrated in FIGURE 29, MTR increases as a function of increasing pH.

FIGURE 30 illustrates the effect of pH on MT images obtained while saturating the bound water of the Eu(2)⁻ complex (62.5 mM) at 57 ppm, at pH 7.4 and pH 7.8, respectively. These data also illustrate that the NMR signal from the ligand, for example at about 21 ppm, are clearly visible and therefore may be used to determine the concentration of CA agent present in a sample. Because the lanthanide-macrocylic complexes of the

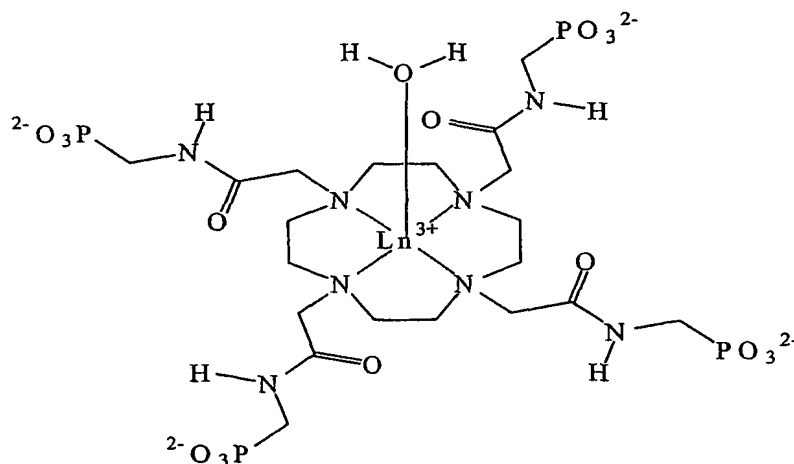
present invention contain MR proton signals outside of the frequency range normally observed for biological molecules, it is possible to determine the concentration of such CAs in biological samples by measuring their ^1H NMR spectra. It follows therefore, that by measuring the CA's concentration, environmental parameters, such as pH, may be determined by analyzing MT image intensities and applying MT theory, as presented in equations 1 and 2, for example. In comparison, the ^1H NMR signals from conventional CA, containing Gd^{3+} for example, are not visible and therefore can not be used as concentration markers.

Experiment 14

In Experiment 14 several other lanthanide-macrocylic complexes of the present invention were synthesized. For example, a lanthanide-macrocylic complex of the present invention was prepared having the general formula, $\text{Ln}(3)^{3+}$, where the four pendent arms R, R', R'' and R''' are all phosphonate diethyl ester-acetamidoacetate (i.e., LnDOTA-4AmPE^{3+}), as depicted below:

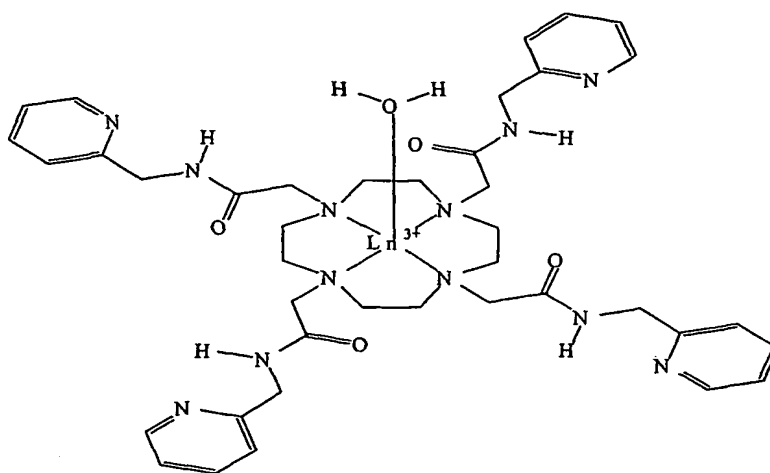


Another lanthanide-macrocylic complex of the present invention was prepared having the general formula, $\text{Ln}(4)^{5-}$, where the four pendent arms R, R', R'' and R''' are all phosphonate-acetamidoacetate (i.e., LnDOTA-4AmP^{5-}), as depicted
 5 below:

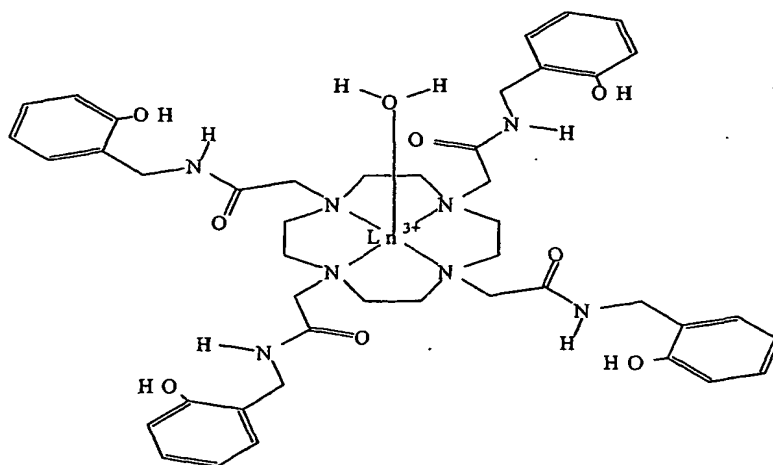


Yet another lanthanide-macrocylic complex of the present invention was prepared having the general formula, $\text{Ln}(5)^{3+}$, where the four pendent arms R, R', R'' and R''' are all pyridine-acetamidoacetate (i.e., LnDOTA-4AmPy^{3+}), as depicted
 10 below:

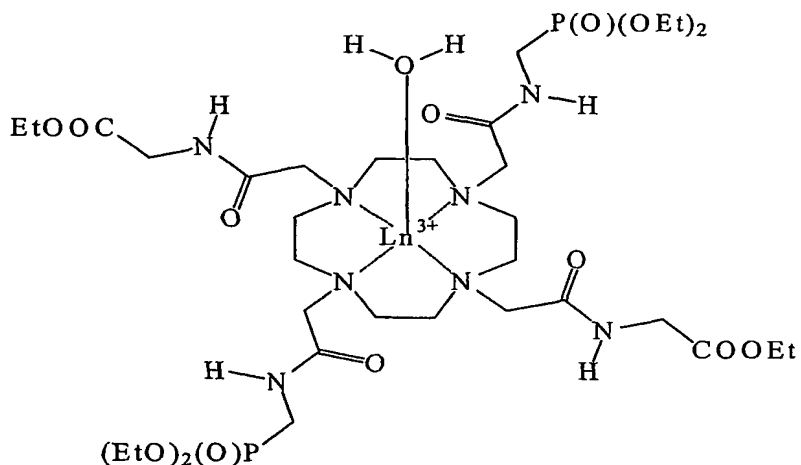
15 Still another lanthanide-macrocylic complex of the



present invention was prepared having the general formula,
 5 $\text{Ln}(6)^{3+}$, where the four pendent arms R, R', R'' and R''' are
 all phenol-acetamidoacetate (i.e., $\text{LnDOTA-4AmPhOH}^{3+}$), as
 depicted below:

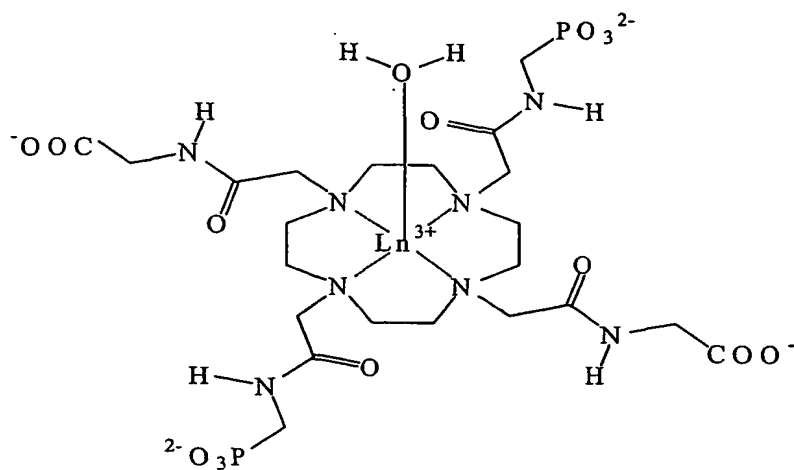


Another lanthanide-macrocylic complex of the present invention was prepared having the general formula, $\text{Ln}(7)^{3+}$, where the two pendent arms R' and R'' are phosphonate diethyl ester-acetamidoacetate and the other two pendent arms R and R' are carboxyethyl-acetamidoacetate (i.e., $\text{LnDOTA-2AmCE-2AmPE}^{3+}$), as depicted below:



Another lanthanide-macrocylic complex of the present invention was prepared having the general formula, $\text{Ln}(8)^{3-}$, where the two pendent arms R' and R'' are phosphonate-acetamidoacetate and the other two pendent arms R and R' are carboxyl-acetamidoacetate (i.e., $\text{LnDOTA-2AmC-2AmP}^{3-}$), as depicted below:

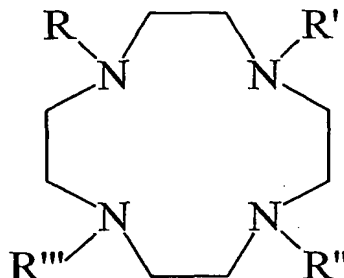
15



Although the present invention has been described in detail, those skilled in the art should understand that they
5 can make various changes, substitutions and alterations herein without departing from the spirit and scope of the invention in its broadest form.

WHAT IS CLAIMED IS:

1. A contrast agent comprising:
 a tetraazacyclododecane ligand having a general formula
 5 as follows:



- wherein pendent arms R, R', R'' and R''' are amides having a general formula: $-CR_1H-CO-NH-CH_2-R_2$, wherein R_1 includes organic substituents and R_2 is not hydrogen; and
 10 a paramagnetic metal ion coordinated to said tetraazacyclododecane ligand.

2. The contrast agent as recited in Claim 1 further including a water molecule associated with said
 15 tetraazacyclododecane ligand and said paramagnetic metal ion such that said water molecule has a $\Delta\omega \cdot \tau_M \geq 1$.

3. The contrast agent as recited in Claim 2 wherein
 said $\Delta\omega \geq 6$ ppm.

4. The contrast agent as recited in Claim 2 wherein said $\tau_M \geq 1 \mu s$.

5. The contrast agent as recited in Claim 1 wherein said
5 paramagnetic metal is selected from the group consisting of:

Eu³⁺;

Tb³⁺;

Dy³⁺; and

Ho³⁺.

10

6. The contrast agent as recited in Claim 1 wherein said
paramagnetic metal is selected from the group consisting of:

Pr³⁺;

Nd³⁺;

15

Sm³⁺;

Er³⁺; and

Tm³⁺.

7. The contrast agent as recited in Claim 1 wherein said
20 R₂ does not have a proton exchangeable group.

8. The contrast agent as recited in Claim 7 wherein
said R₂ is selected from the group consisting of:

Alkyl groups having 20 carbon atoms or less;

25

Cycloalkyl groups having 20 carbon atoms or less;

Alkyloxy groups having 20 carbon atoms or less;

Alkyl ethers having 10 oxygen atoms or less and 20 carbon
atoms or less; and

Polyols having 20 carbon atoms or less.

30

9. The contrast agent as recited in Claim 1 wherein said R_1 is selected from the group consisting of:

H;

Alkyl groups having 20 carbon atoms or less;

5 Cycloalkyl groups having 20 carbon atoms or less;

Alkyloxy groups having 20 carbon atoms or less;

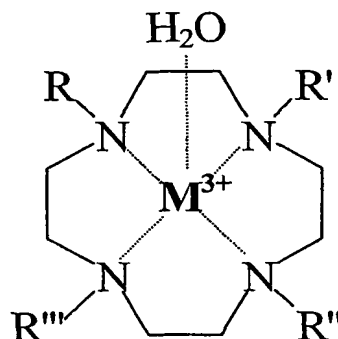
Alkyl ethers having 10 oxygen atoms or less and 20 carbon atoms or less; and

Polyols having 20 carbon atoms or less.

10

10. A method of using a magnetic resonance (MR) contrast agent, comprising:

subjecting a contrast agent contained within a sample to a radio frequency pulse wherein said contrast agent is a
15 tetraazacyclododecane ligand having a general formula of:



wherein pendent arms R, R', R'' and R''' comprise organic substituents and said tetraazacyclododecane ligand further includes a paramagnetic metal ion (M^{3+}) coordinated to
20 said tetraazacyclododecane ligand and a water molecule (H_2O) associated with said tetraazacyclododecane ligand; and

obtaining a magnetization transfer signal by applying a radio frequency pulse at a resonance frequency of said water molecule.

25

11. The method as recited in Claim 10 wherein said water molecule has a $\Delta\omega \cdot \tau_M \geq 1$.

12. The method as recited in Claim 10 further includes
5 producing a magnetization transfer magnetic resonance image from said magnetization transfer signal.

13. The method as recited in Claim 10 further include
10 applying said radio frequency pulse as a saturating pulse.

14. The method as recited in Claim 10 further includes
said contrast agent with at least one pendent arm containing an amide group.

15 15. The method as recited in Claim 14 wherein said pendent arms are identical and have the general formula:
-CHR₁-CO-NR₂-R₃, wherein R₁, R₂ and R₃ comprise organic substituents.

20 16. The method as recited in Claim 14 wherein said R₁ and R₂ are H, and R₃ has the general formula: -(CH₂)_nCOOR₄ where

n = 1-20; and

R₄ is selected from the group consisting of:

25 H;
Group IA or IIA metal ions; and
alkyl groups containing from one to twenty Carbon atoms.

17. The method as recited in Claim 14 wherein said paramagnetic metal ion is selected from the group consisting of:

5 Tb^{3+} ;
 Dy^{3+} ; and
 Ho^{3+} .

18. The method as recited in Claim 14 wherein said paramagnetic metal ion is selected from the group consisting of:

10 Eu^{3+} ;
 Pr^{3+} ; and
 Nd^{3+} .

19. The method as recited in Claim 14 wherein said R_1 and R_2 are H, and R_3 has the general formula: -

$(CH_2)_n P(O)(OR_4 OR_5)$ where

$n = 1-20$;

said R_4 is selected from the group consisting of:

20 H;
 alkaline earth metal ions of Groups IA or IIA; and
 alkyl groups containing one to twenty Carbon atoms;
and said R_5 is selected from the group consisting of:

25 H;
 alkaline earth metal ions of Groups IA or IIA; and
 alkyl groups containing one to twenty Carbon atoms.

20. The method as recited in Claim 14 wherein said

R₁ and R₂ are H, and R₃ has the general formula: $-(CH_2)_nR_4$ where
n = 1-20; and

R₄ is selected from the group consisting of:

Pyridine (Py); and

5 Phenol (Ph).

21. The method as recited in Claim 14 wherein said
pendent arms R and R'' are identical, said pendent arms R' and
R''' are identical, and said pendent arms R' and R''' are not
10 equal to said pendent arms R and R''.

22. The method as recited in Claim 21 wherein
said pendent arms R and R'' have the general formula:

-CR₁H-CO-NH-CH₂-R₂; and

15 said pendent arms R' and R''' have the general formula:

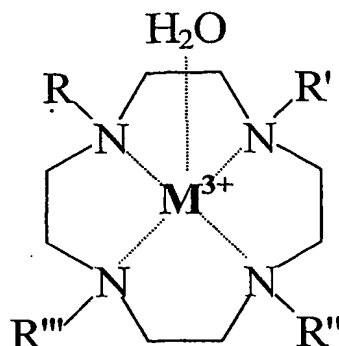
-CHR₃-CO-NH-R₄ wherein

said R₁, R₂, R₃, and R₄ comprise organic substituents; and
R₂ is not equal to R₄.

20 23. The method as recited in Claim 14 further
includes obtaining said magnetization transfer signal by
applying a radio frequency pulse at a resonance frequency of
said protons associated with said amide.

25 24. A magnetic resonance system, comprising:

a magnetic resonance (MR) contrast agent, wherein said MR
agent tetraazacyclododecane ligand, having a general formula
of:



wherein pendent arms R, R', R'' and R''' comprise organic substituents and said tetraazacyclododecane ligand further includes a paramagnetic metal ion (M^{3+}) coordinated to
 5 said tetraazacyclododecane ligand and a water molecule (H_2O) associated with said tetraazacyclododecane ligand, wherein said MR contrast agent produces a magnetization transfer signal when subjected to a radio frequency pulse; and

a magnetic resonance apparatus configured to produce said
 10 frequency pulse.

25. The magnetic resonance system recited in Claim 24, further comprising a sample containing said MR contrast agent.

15 26. The magnetic resonance system recited in Claim 24, wherein said sample is a living subject.

27. The magnetic resonance system recited in Claim 24, wherein said magnetic resonance apparatus produces a magnetization transfer image of said sample from said magnetization transfer signal.

5

28. The magnetic resonance system recited in Claim 27, wherein said magnetic resonance apparatus produces said magnetization transfer image by applying said radio frequency pulse at a resonance frequency of said water molecule.

10

29. The magnetic resonance system recited in Claim 28, wherein said magnetic resonance apparatus produces a magnetization transfer difference image by applying said radio frequency pulse at a $\Delta\omega$ of said water molecule, acquiring said magnetization transfer signal and subtracting said signal from a MR signal obtained by applying a radio frequency pulse at $-\Delta\omega$.

30. The magnetic resonance system recited in Claim 27, wherein said magnetic resonance apparatus produces said magnetization transfer image by applying said radio frequency pulse at a resonance frequency of protons associated with an amide included in one or more of said pendent arms.

31. The magnetic resonance system recited in Claim 24, wherein said radio frequency pulse is produced by said magnetic resonance apparatus and is a saturating pulse.

32. The magnetic resonance system recited in Claim 24, wherein said saturating pulse is applied at a resonance frequency of said water molecule.

33. The magnetic resonance system recited in Claim 24, wherein said saturating pulse ranges from about 1 to about 3 seconds.

5 34. The magnetic resonance system recited in Claim 24 wherein said water molecule has a $\Delta\omega \cdot \tau_M \geq 1$.

35. The magnetic resonance system recited in Claim 24 wherein said $\Delta\omega \geq 6$ ppm.

10

36. The magnetic resonance system recited in Claim 24 wherein said $\tau_M \geq 1 \mu s$.

()

()

100

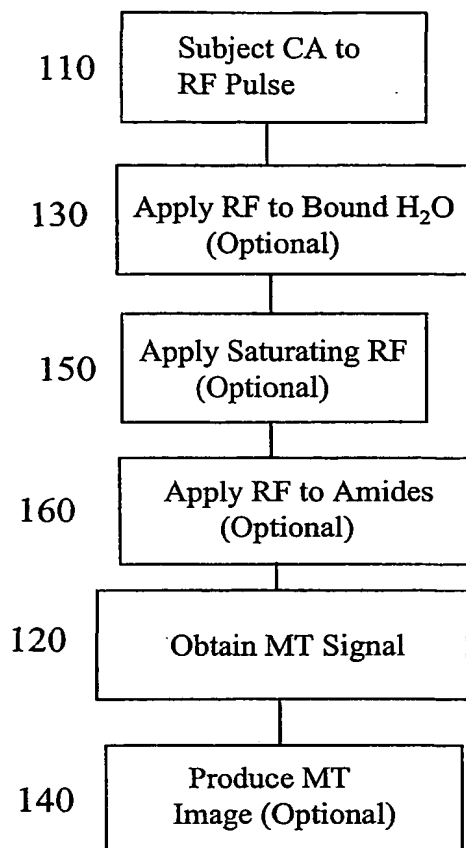


FIGURE 1

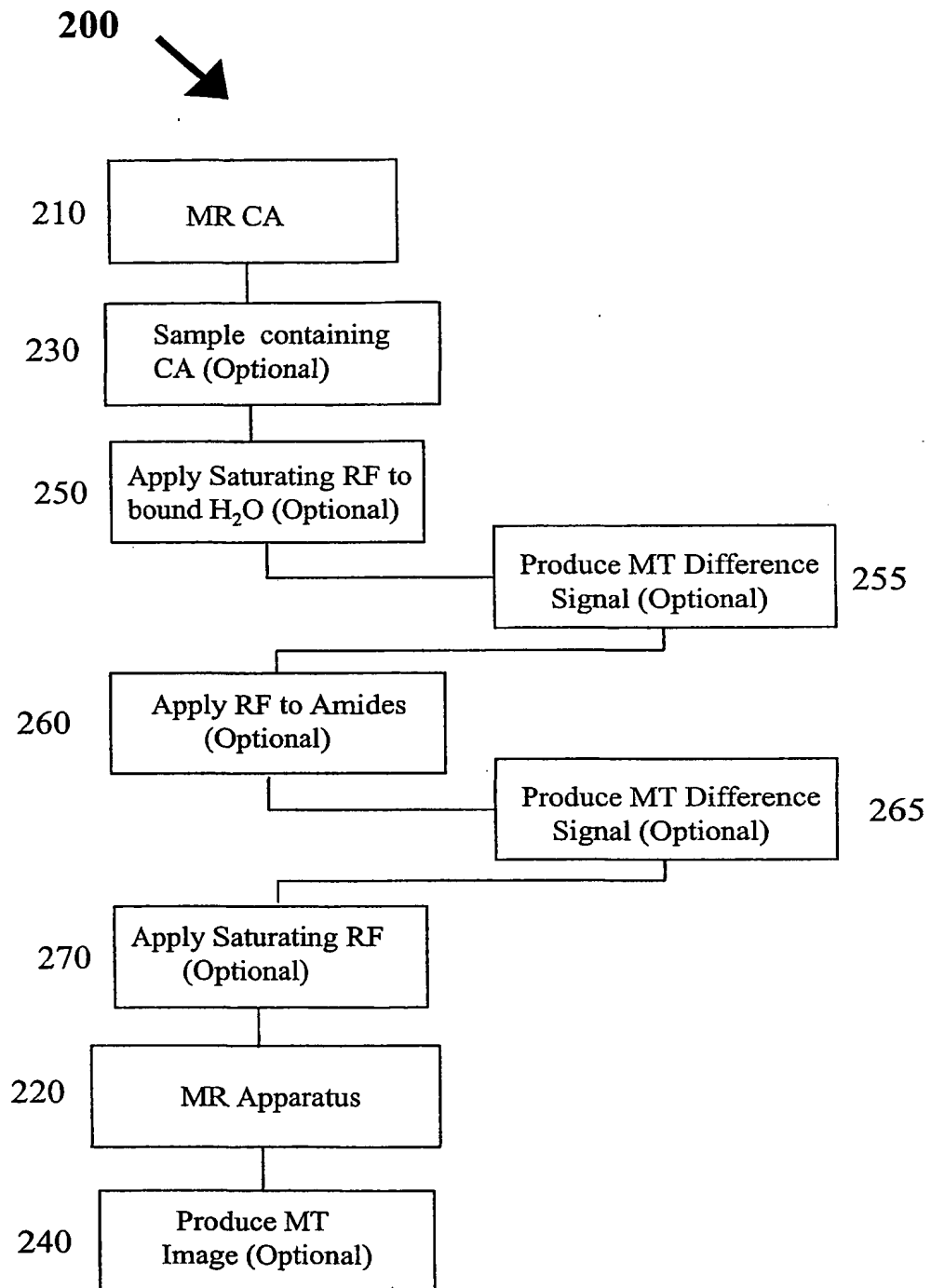


FIGURE 2

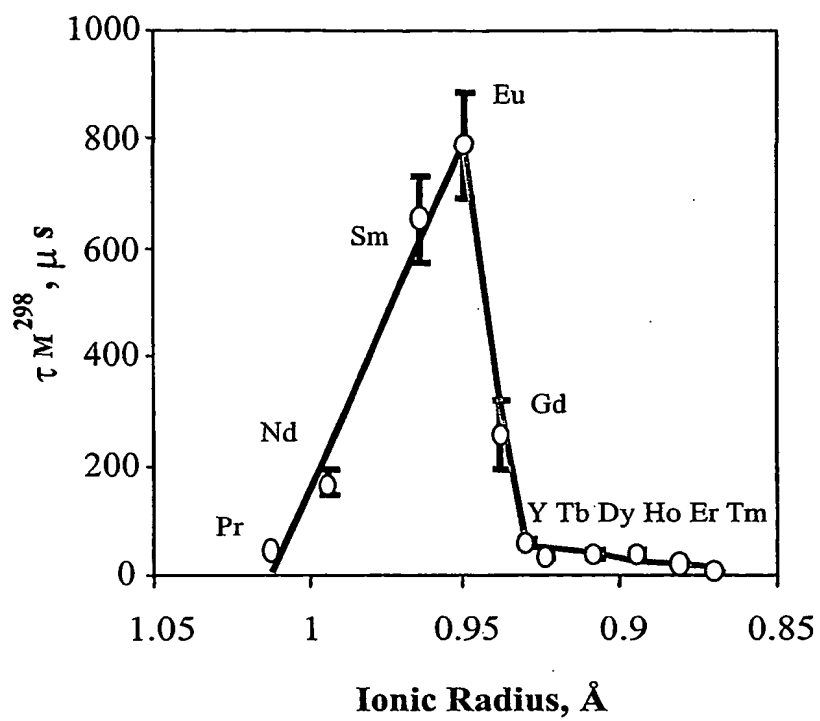


FIGURE 3

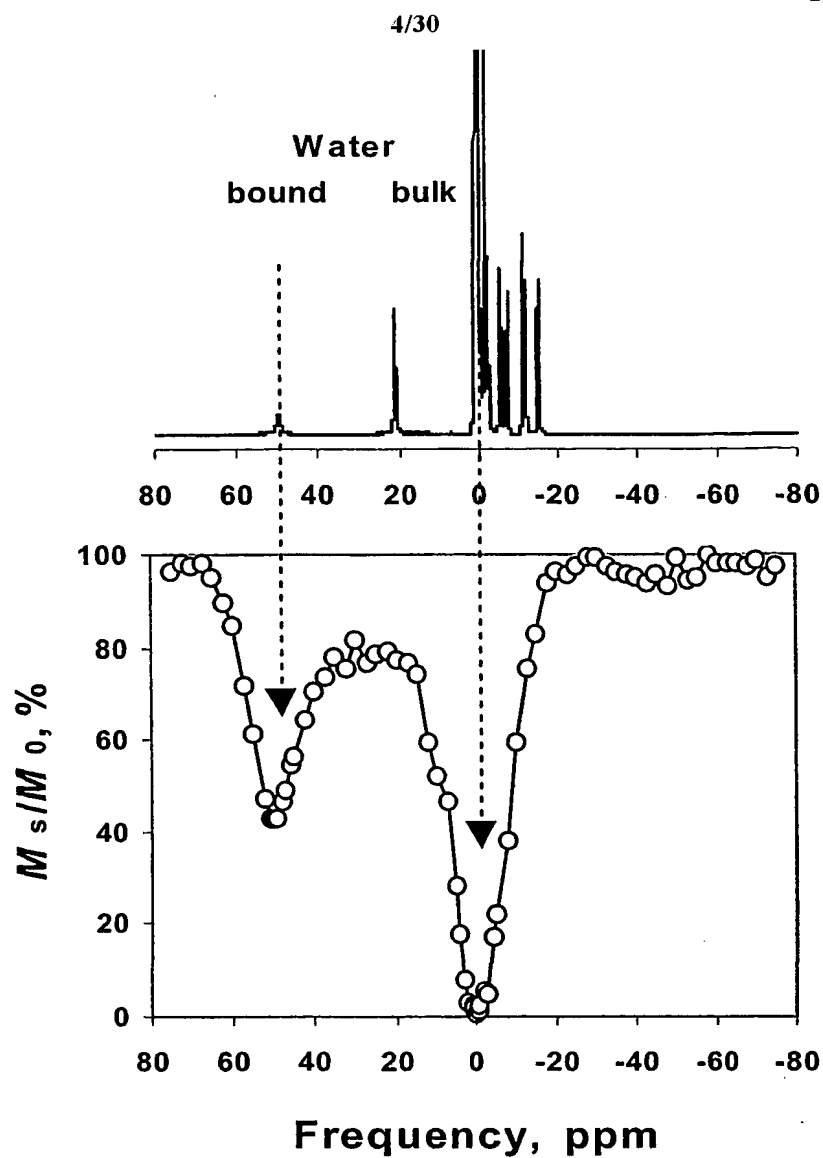


FIGURE 4

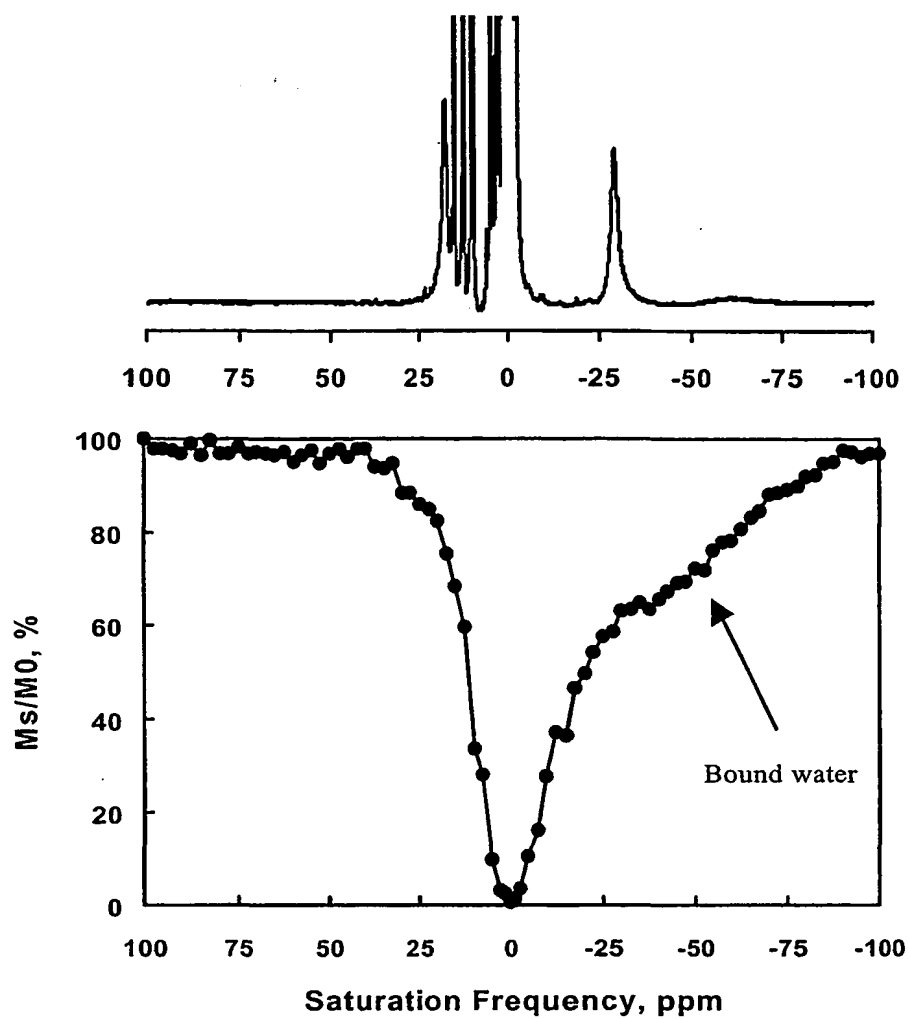


FIGURE 5

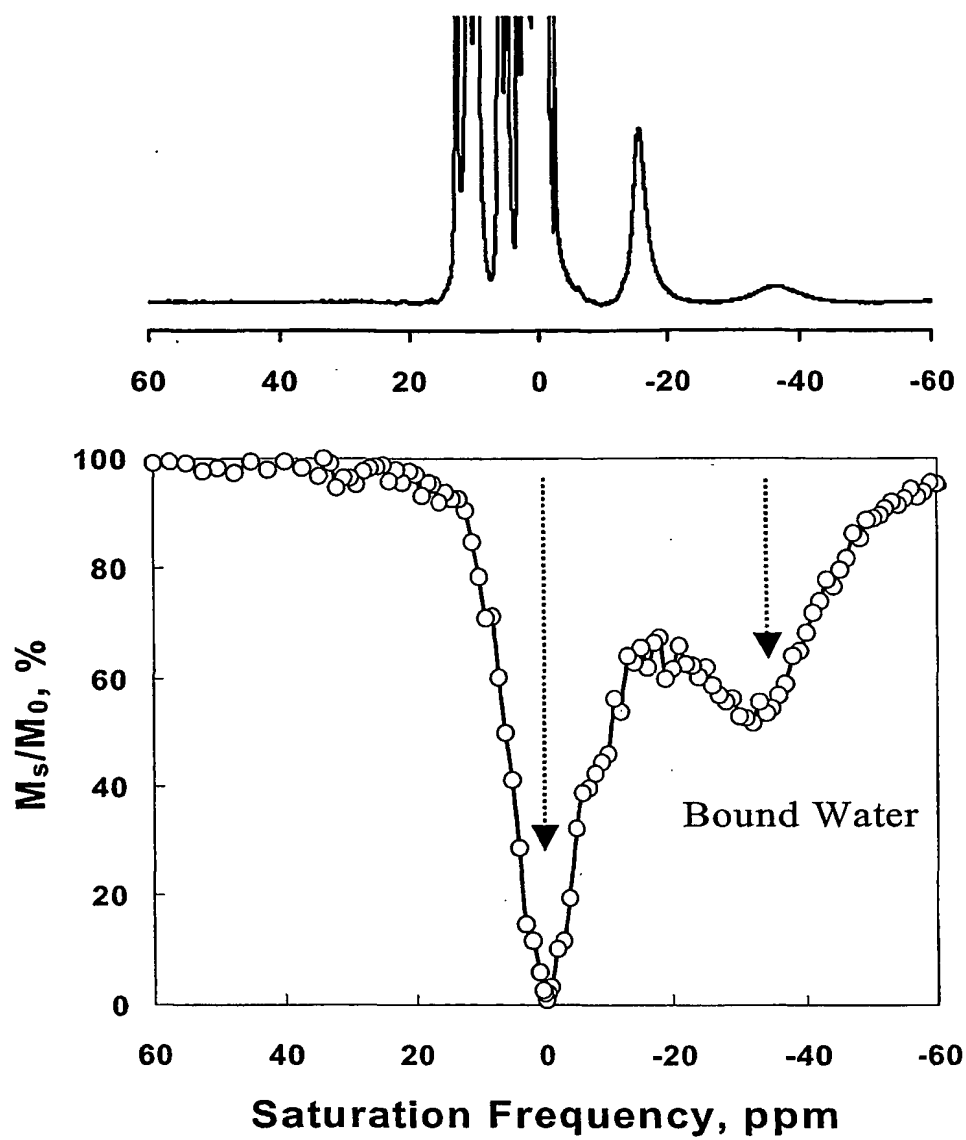


FIGURE 6

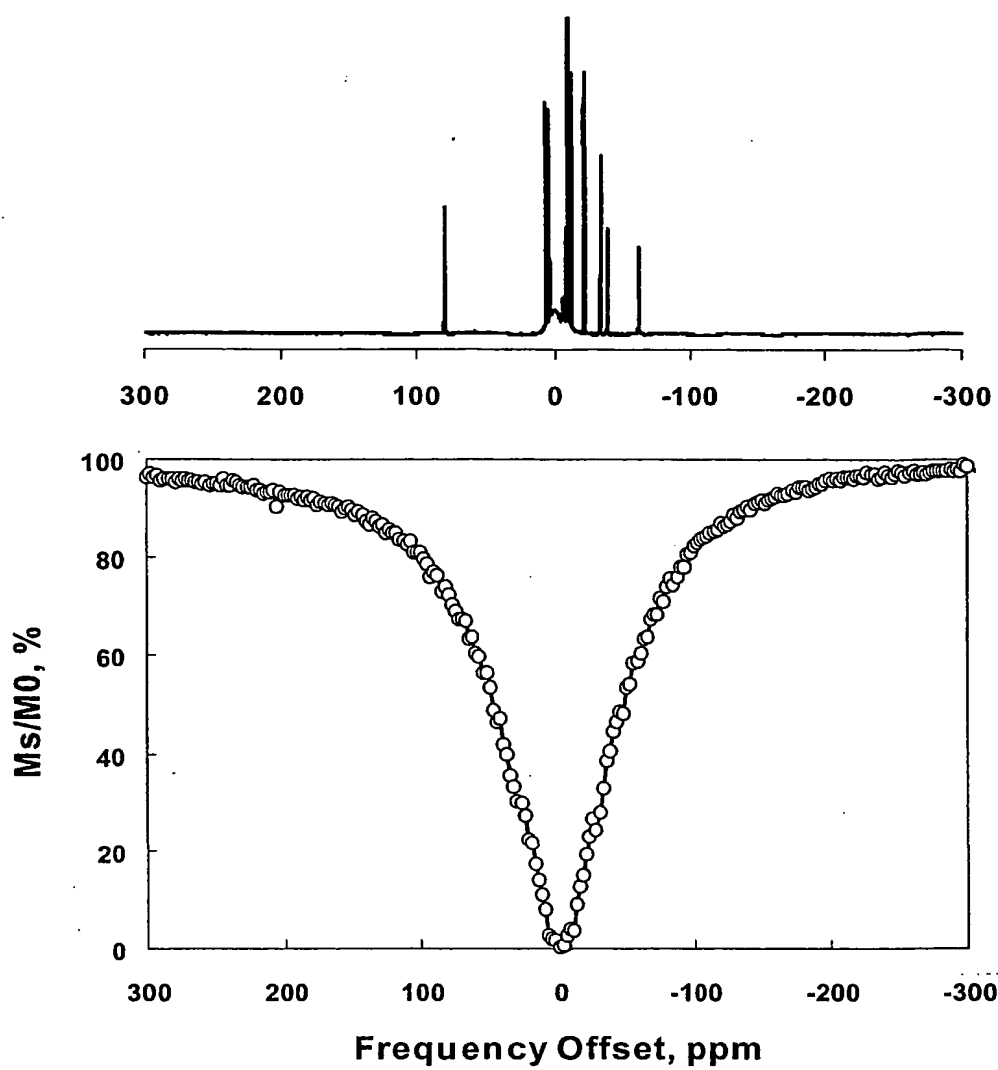


FIGURE 7

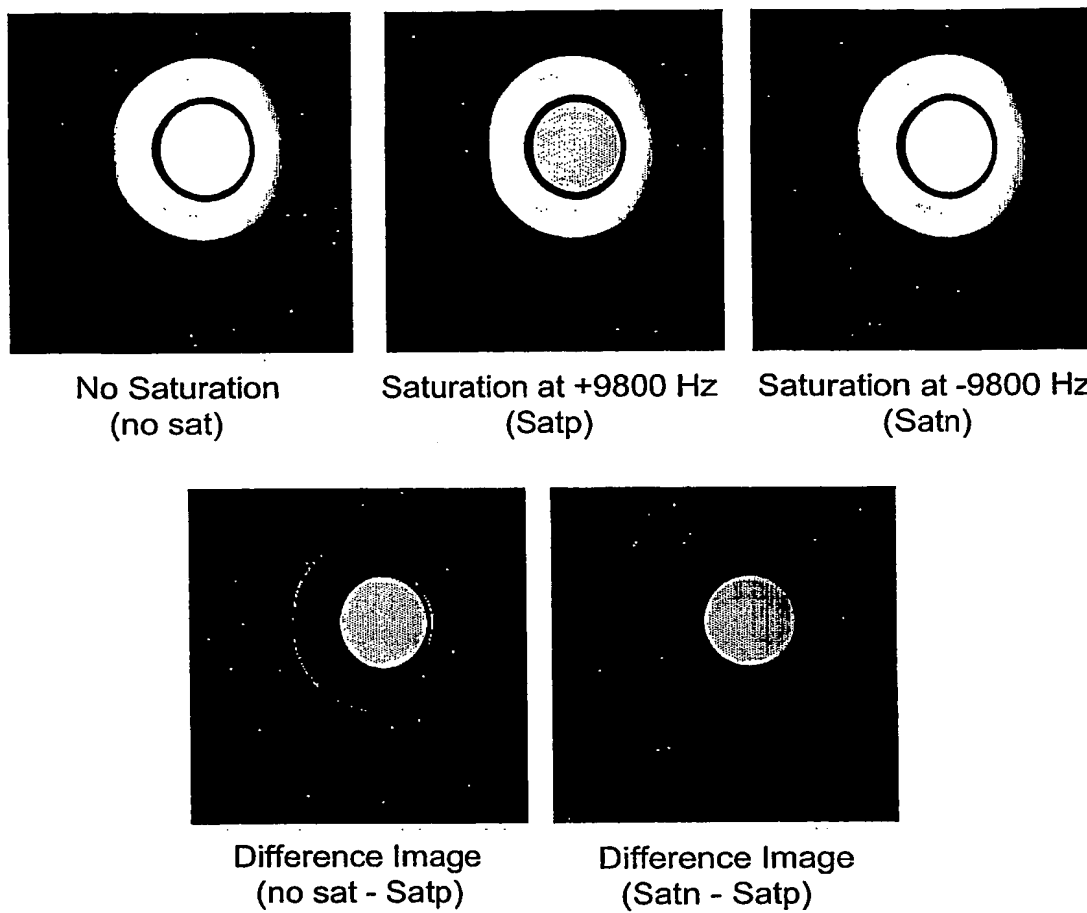


FIGURE 8

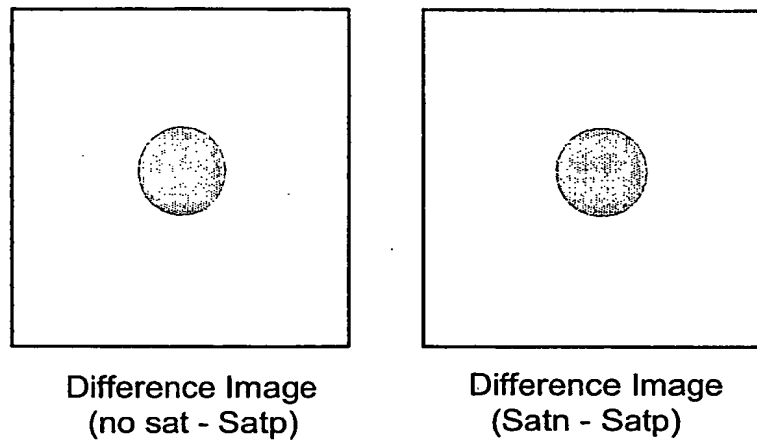
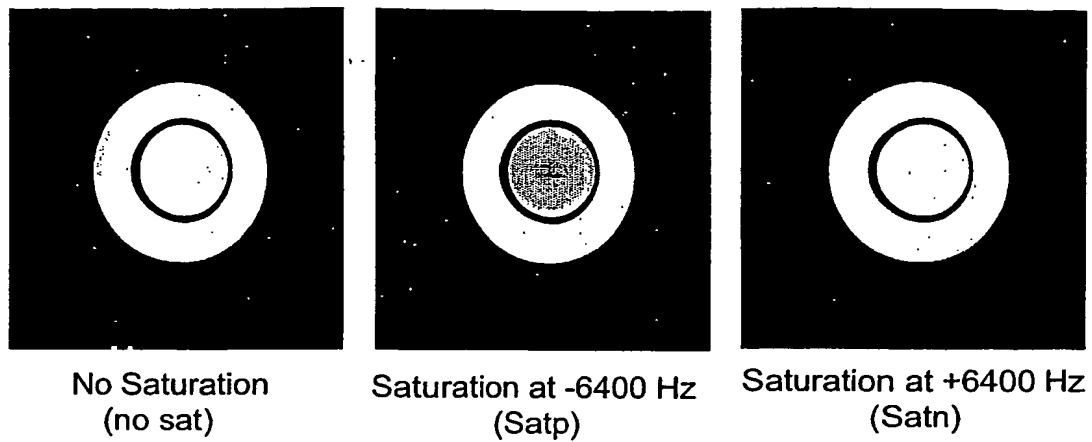


FIGURE 9

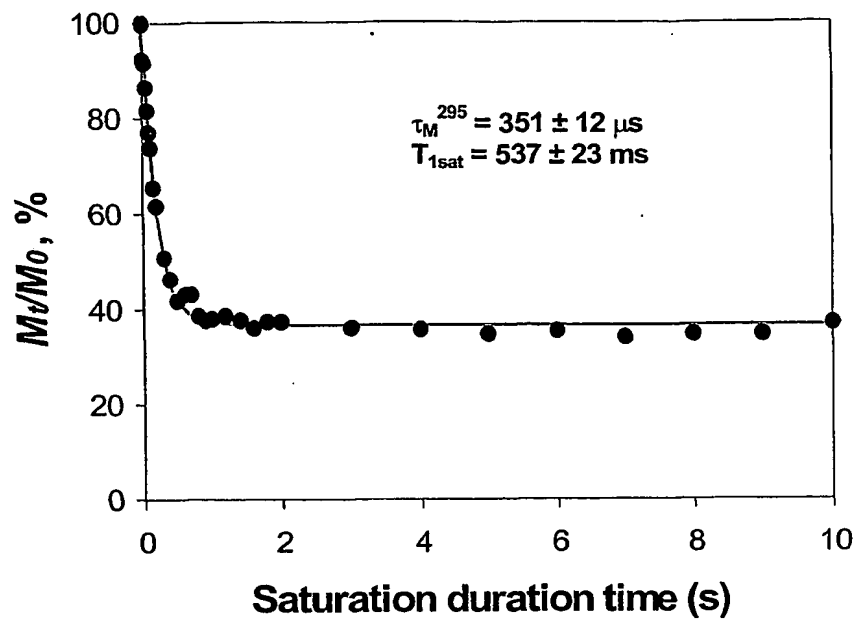


FIGURE 10

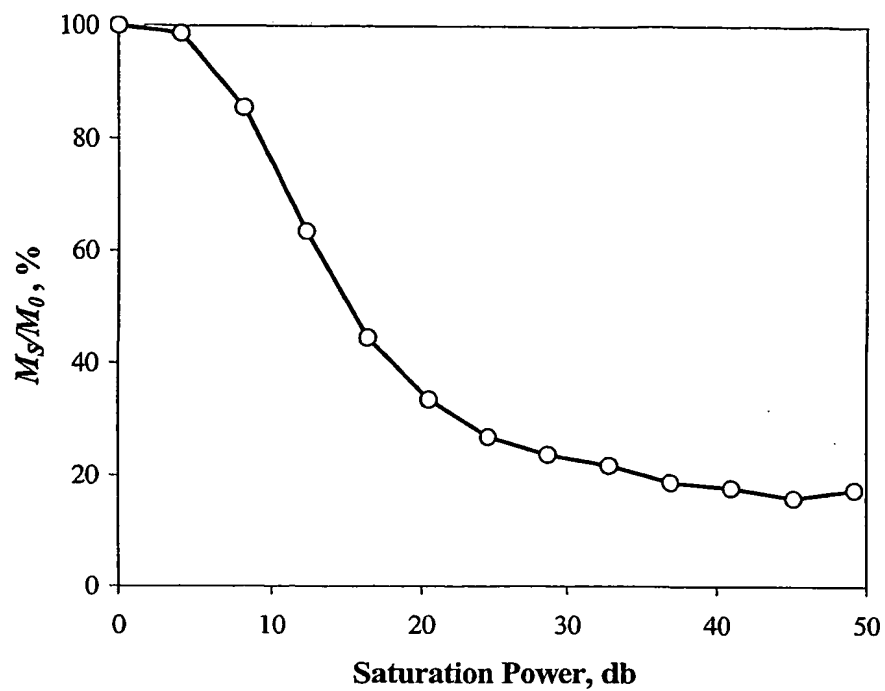


FIGURE 11

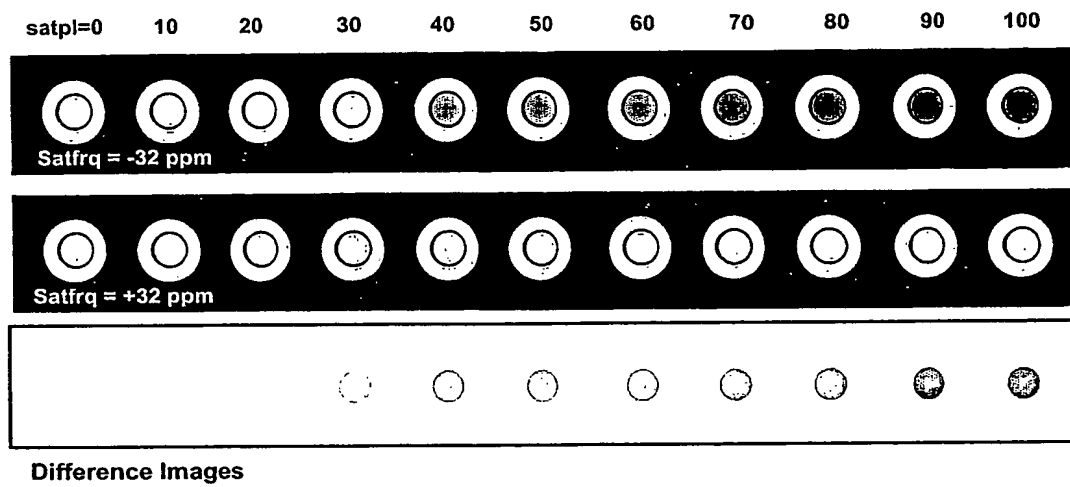


FIGURE 12

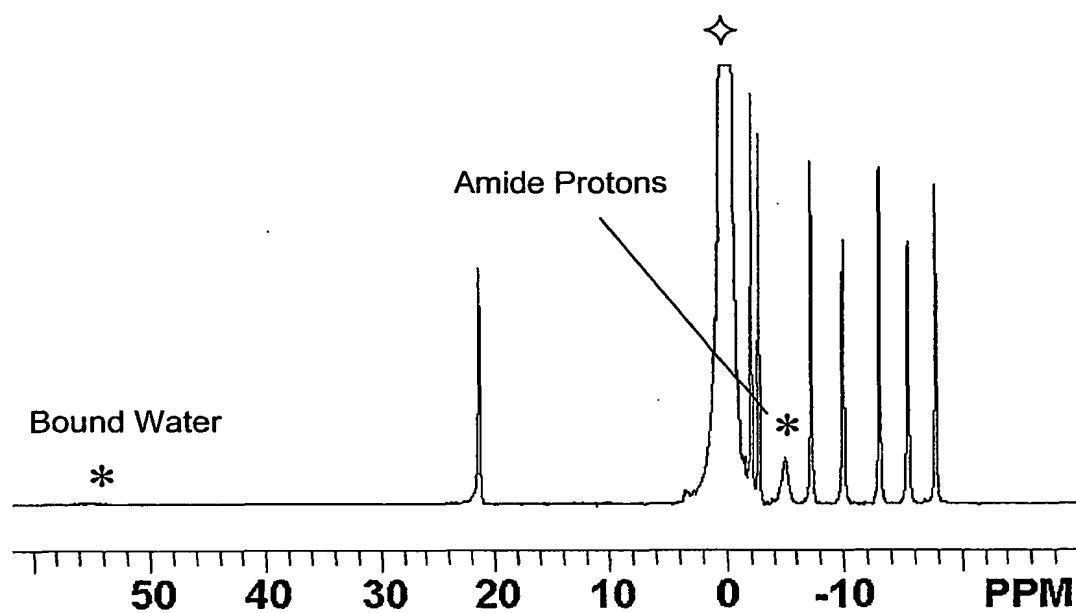


FIGURE 13

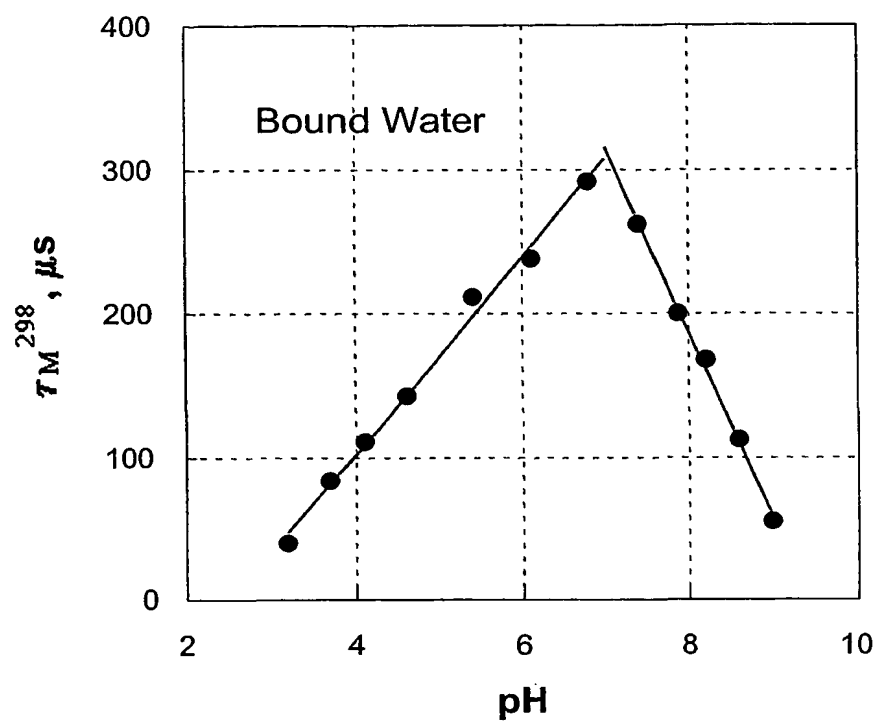


FIGURE 14

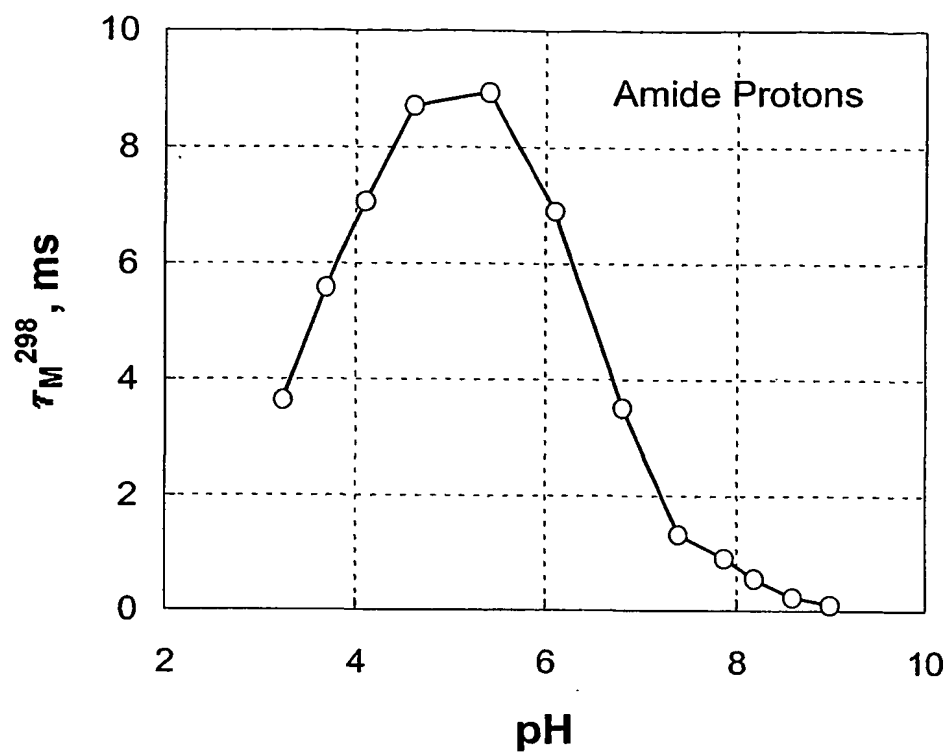


FIGURE 15

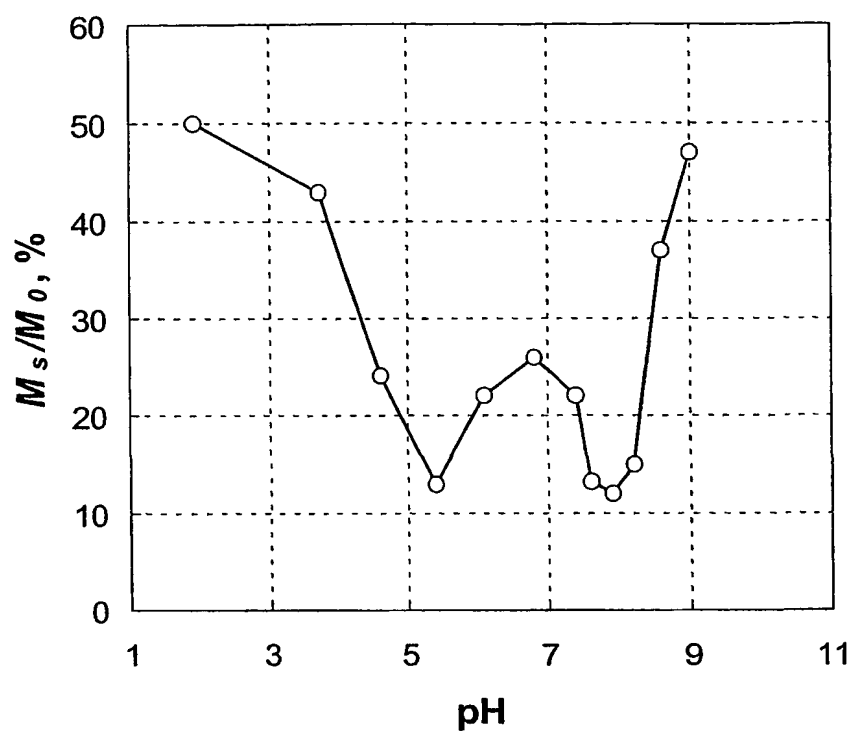


FIGURE 16

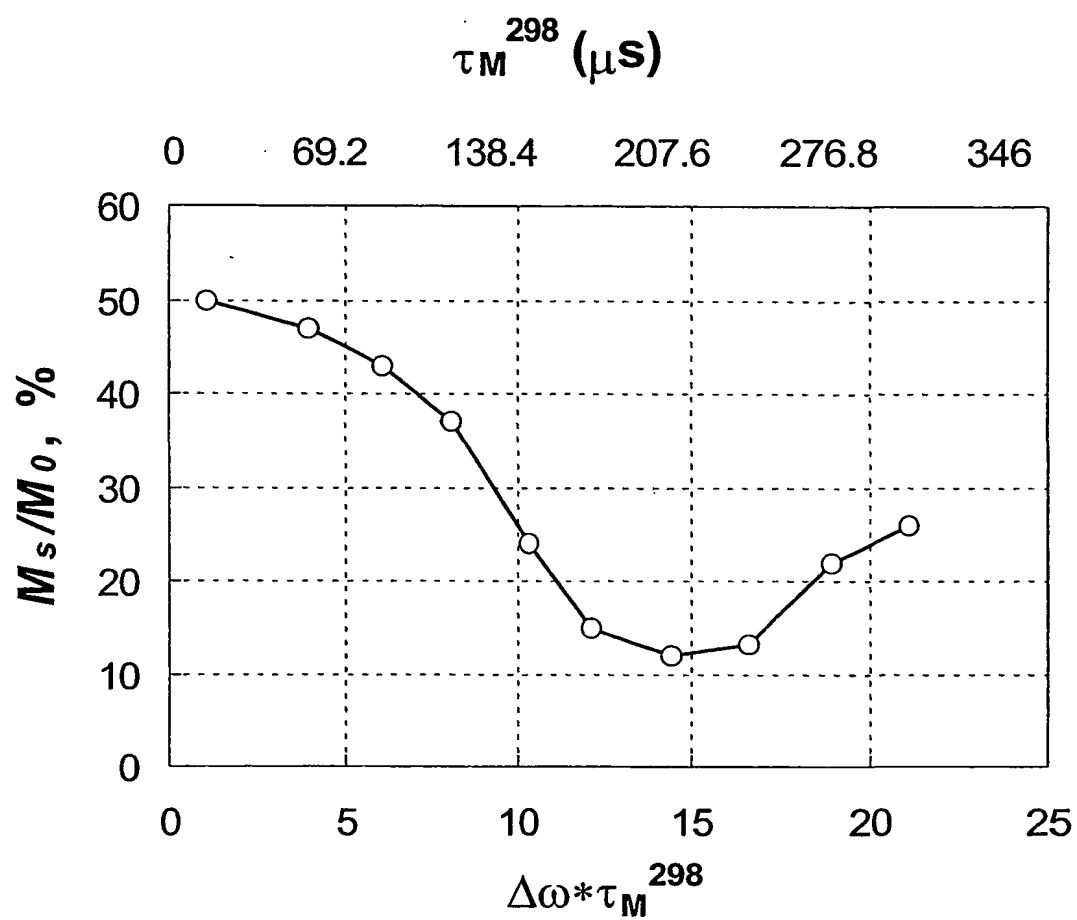


FIGURE 17

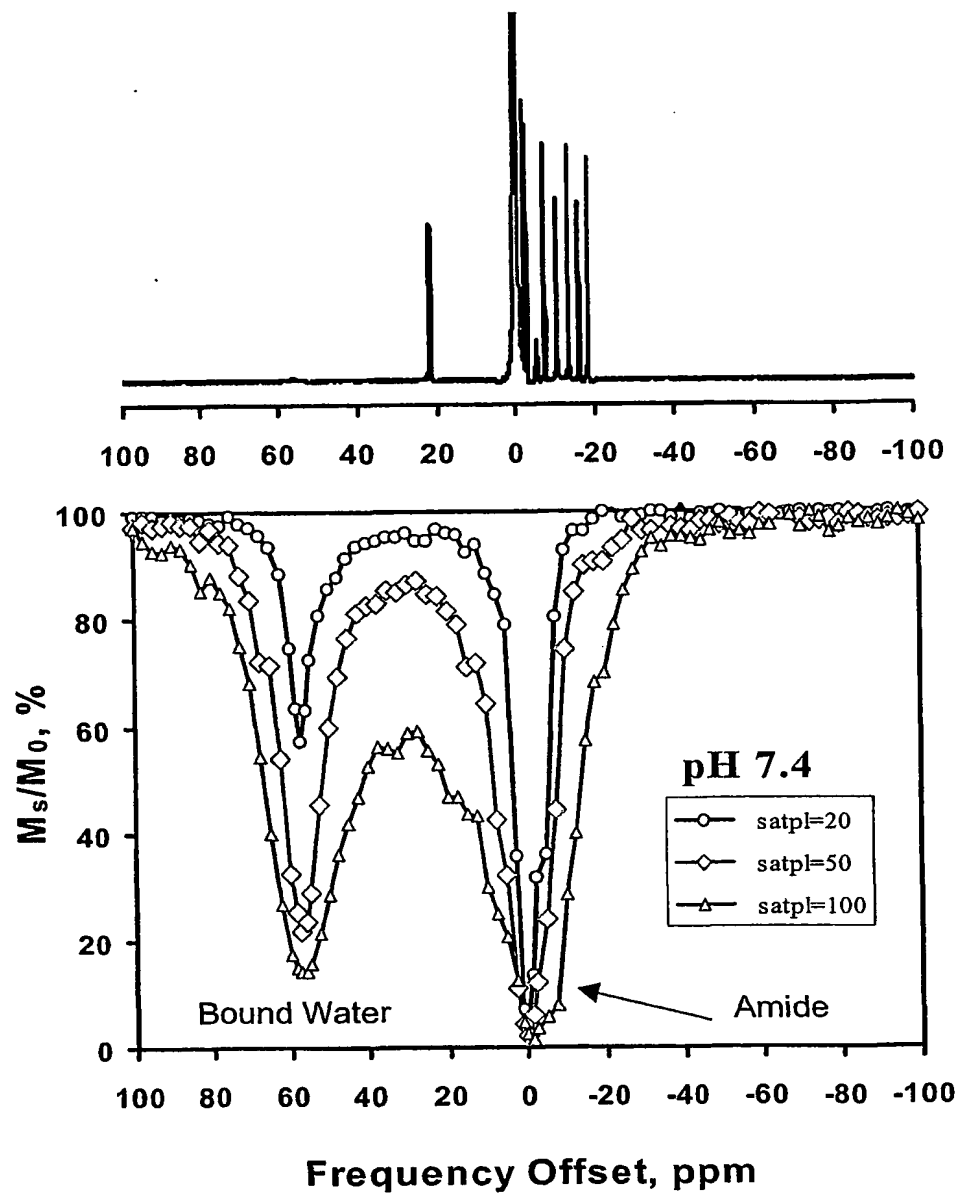


FIGURE 18

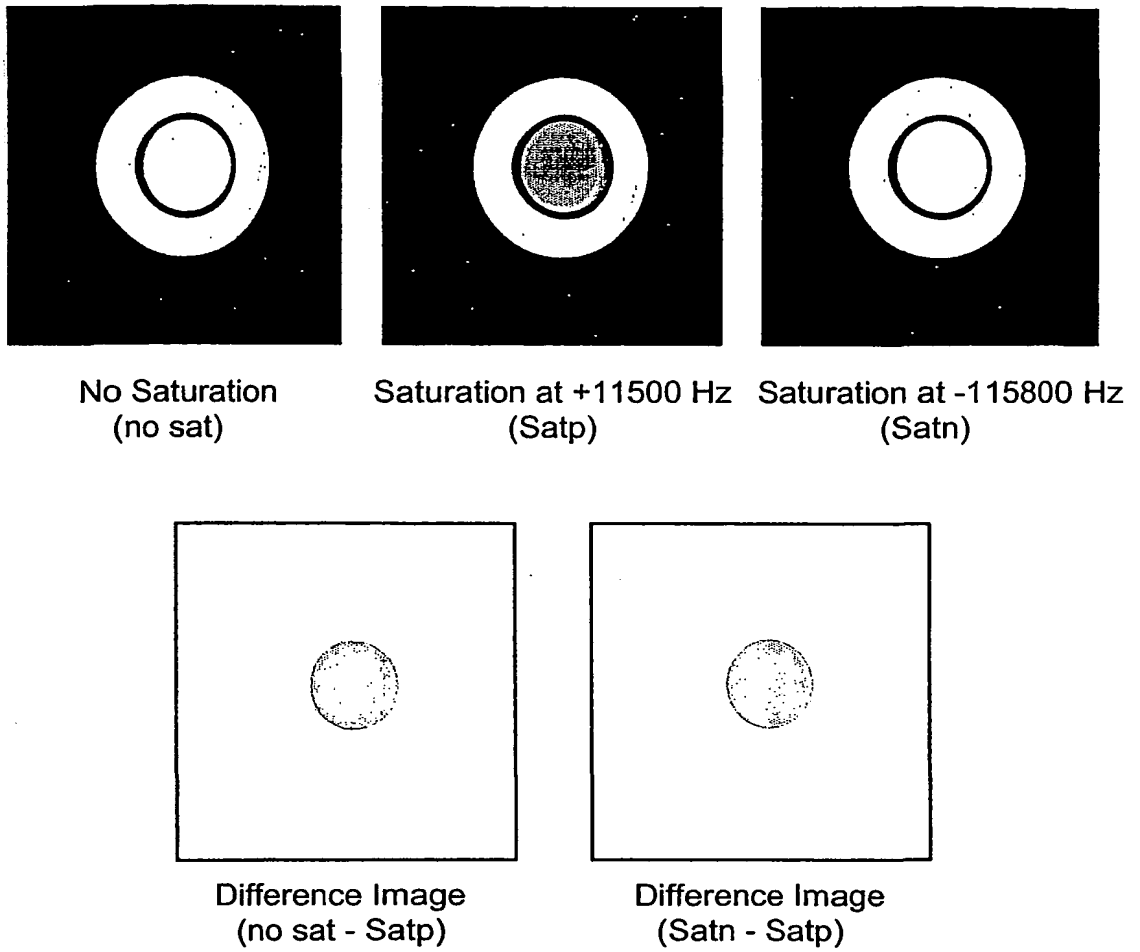


FIGURE 19

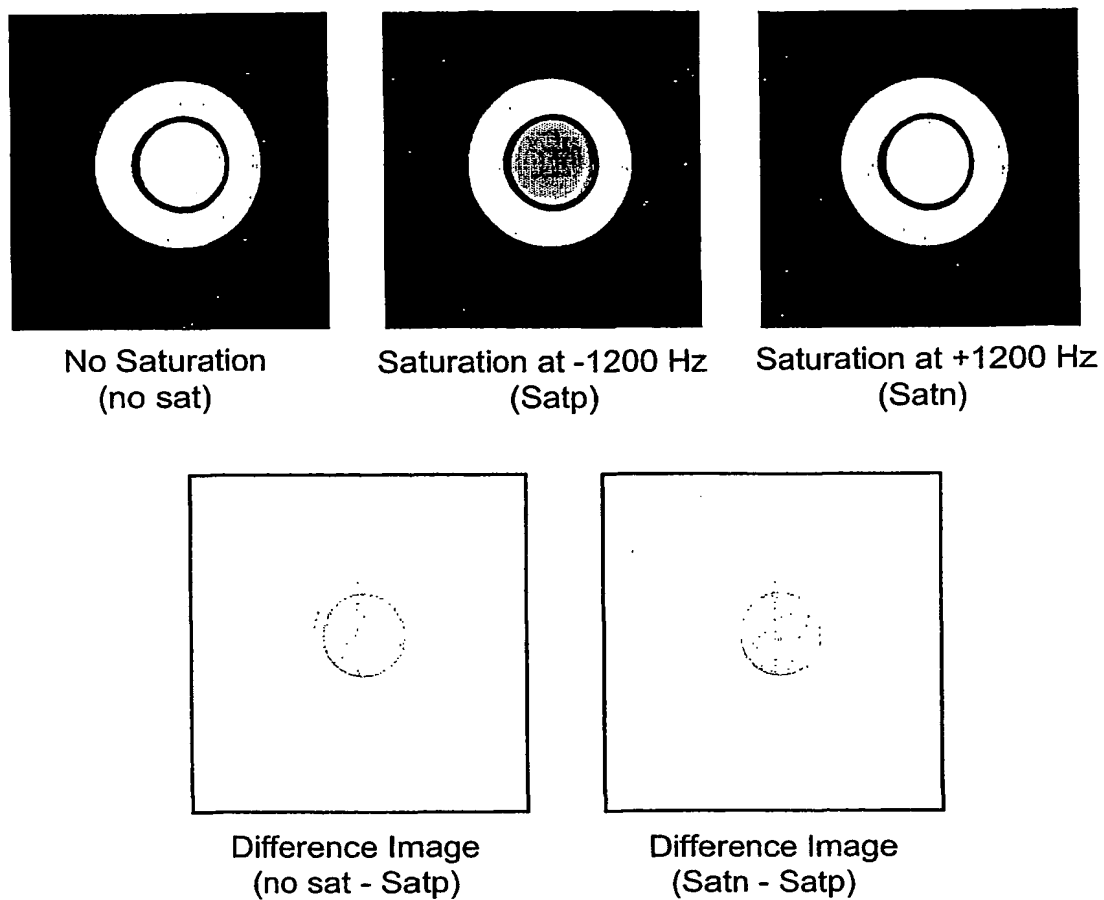
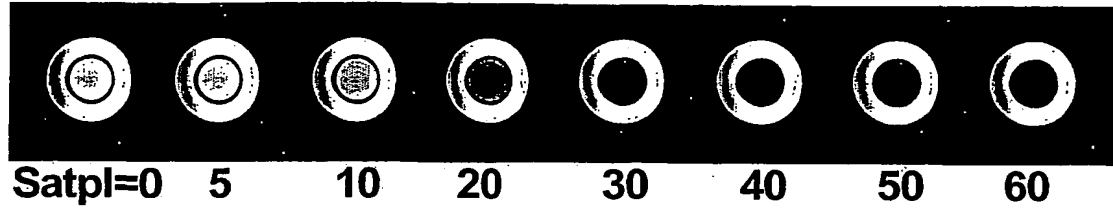
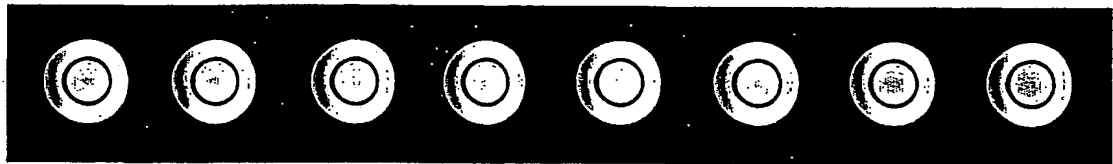


FIGURE 20

a) Satfrq = +11500Hz



b) Satfrq = -11500Hz



c) Difference Images

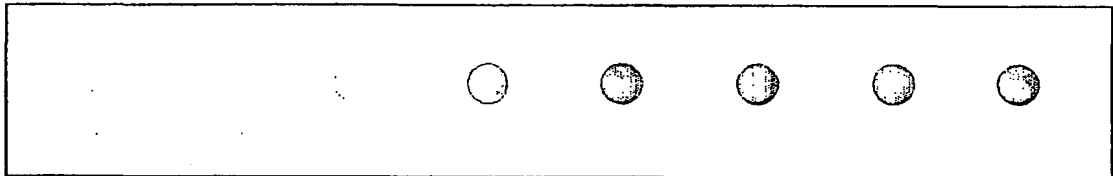
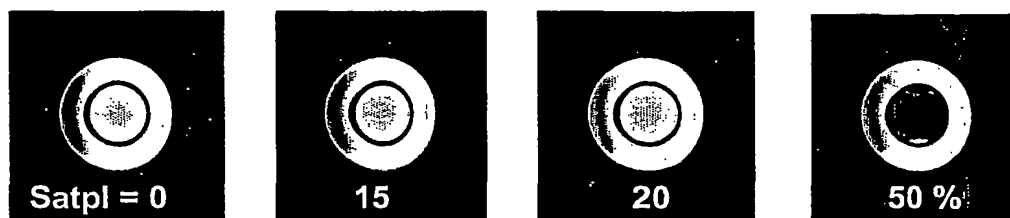
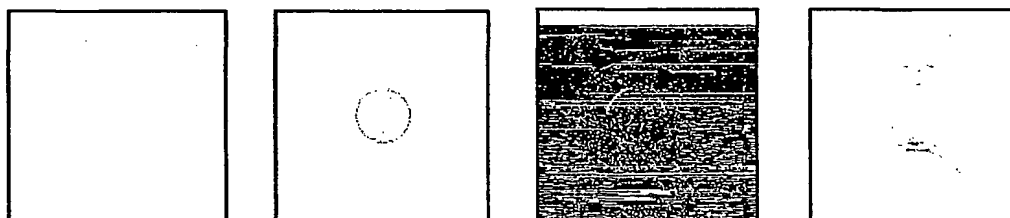


FIGURE 21

a) Satfrq = -1200Hz**b) Satfrq = +1200Hz****c) Difference Images****FIGURE 22**

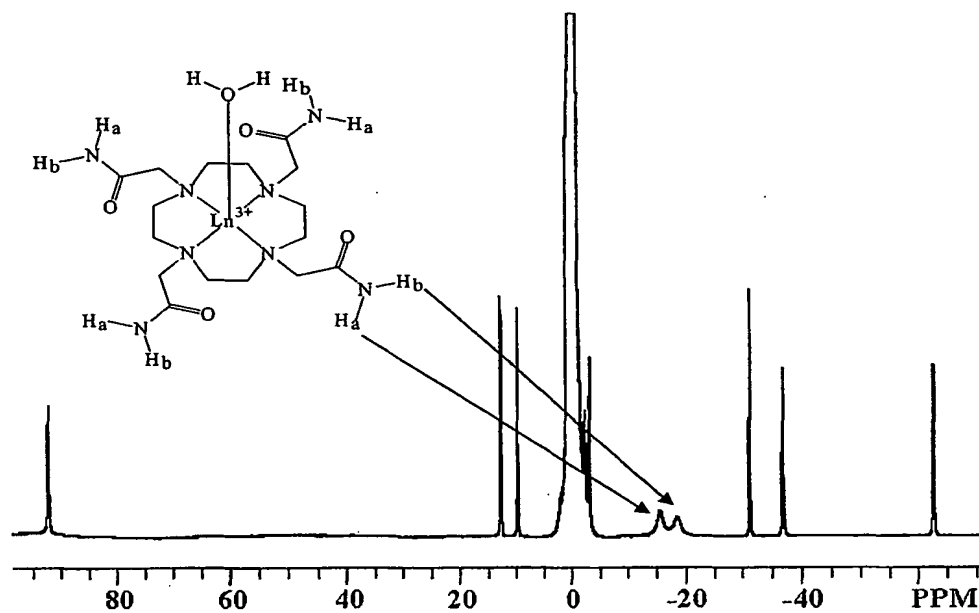


FIGURE 23

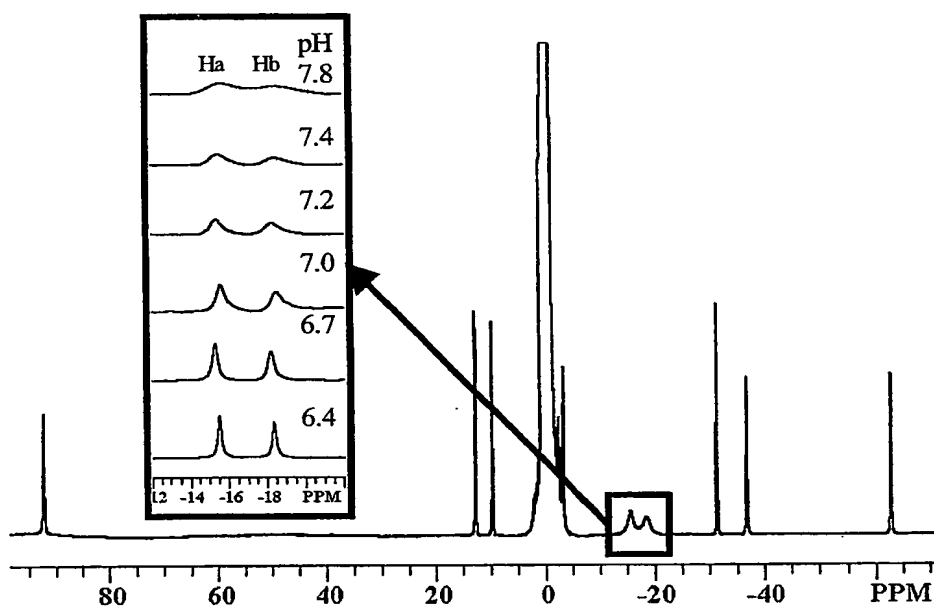


FIGURE 24

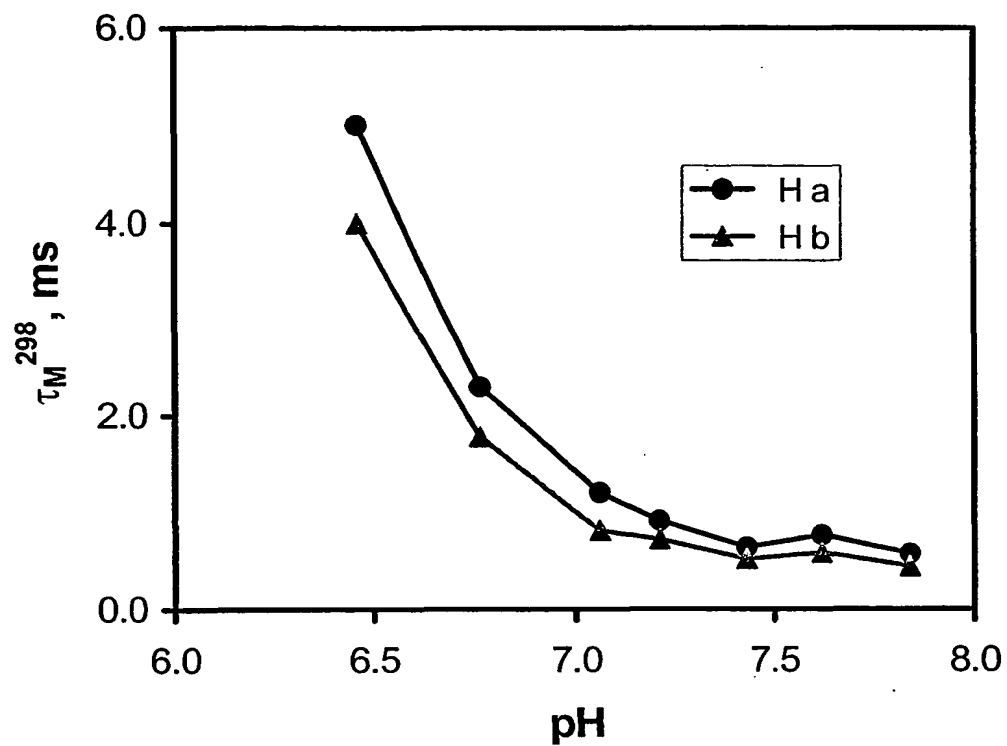


FIGURE 25

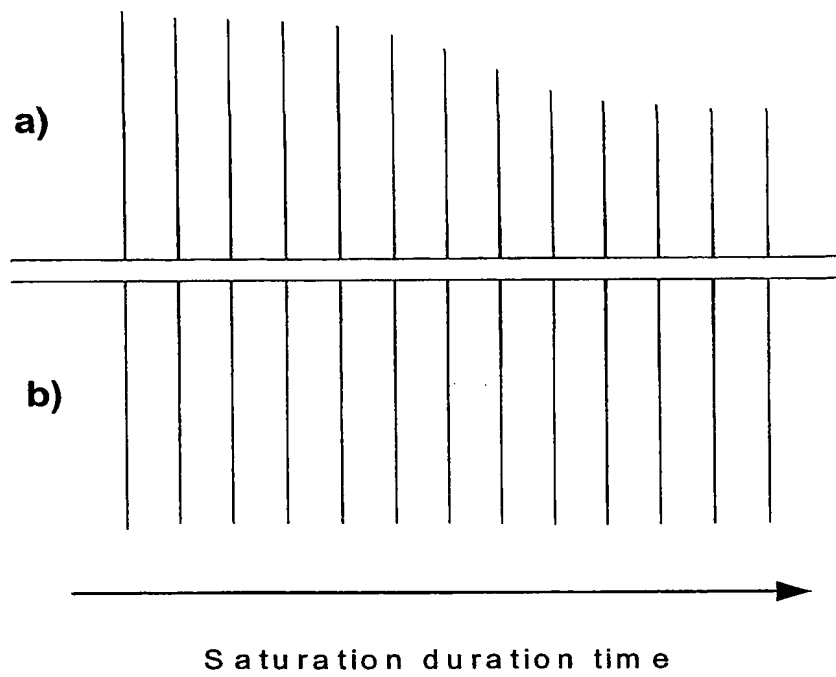


FIGURE 26

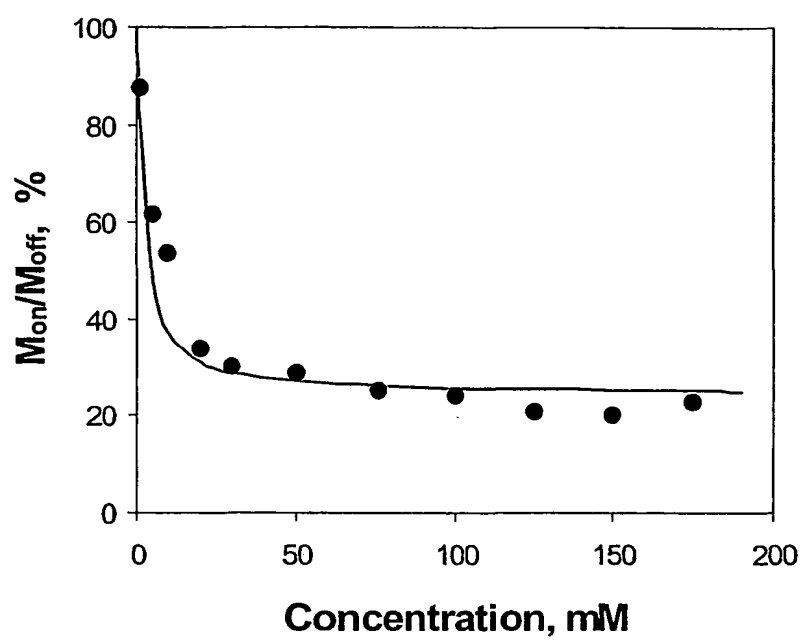


FIGURE 27

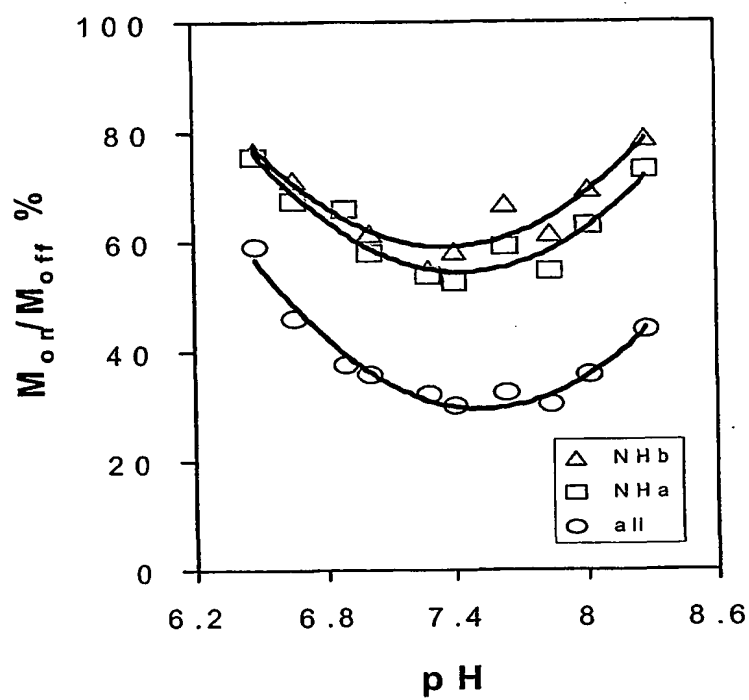


FIGURE 28

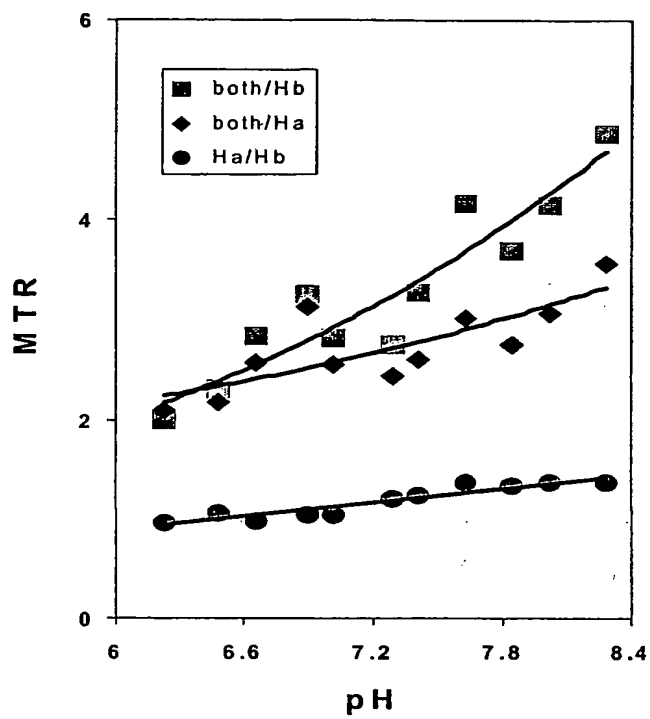


FIGURE 29

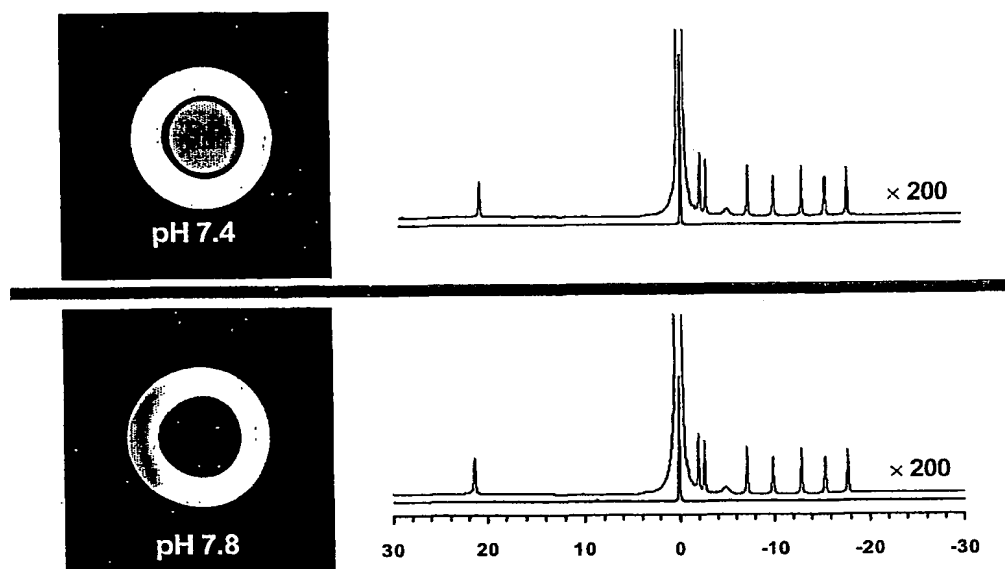


FIGURE 30

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
6 June 2002 (06.06.2002)

PCT

(10) International Publication Number
WO 02/043775 A3

(51) International Patent Classification⁷: A61K 49/10,
G01R 33/28, A61P 43/00

Kuangcong; 2524 Cima Hill Drive, Plano, TX 75023 (US).

(21) International Application Number: PCT/US01/46151

(74) Agent: GAINES, Charles, W.; Hitt Gaines & Boisbrun, PC, P.O. Box 832570, Richardson, TX 75083 (US).

(22) International Filing Date:
20 November 2001 (20.11.2001)

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/252,269 20 November 2000 (20.11.2000) US

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

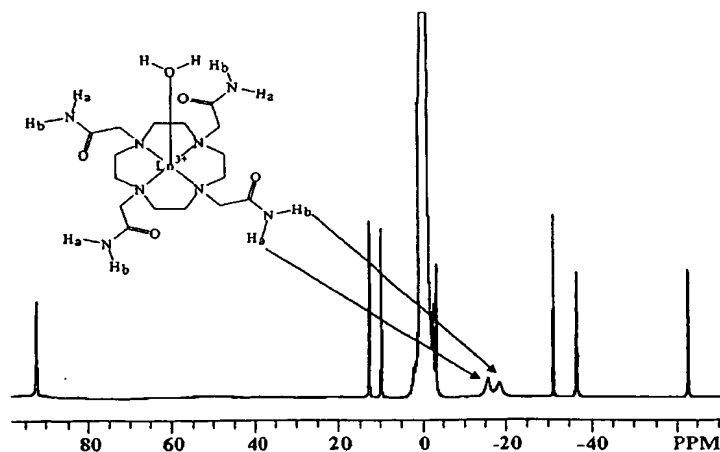
(71) Applicant: BOARD OF REGENTS UNIVERSITY OF TEXAS SYSTEM [US/US]; Gpdfrey, Cullen, M., University of Texas System, 201 W. 7th Street, Austin, TX 78757 (US).

Published:
— with international search report

(72) Inventors: SHERRY, A., Dean; 1634 Spanky Branch Drive, Dallas, TX 75248 (US). ZHANG, Shanrong; 5200 Meadowcreek Drive, #1058, Dallas, TX 75248 (US). WU,

[Continued on next page]

(54) Title: PARAMAGNETIC METAL ION-BASED MACROCYCLIC CONTRAST AGENTS



(57) Abstract: The present invention is directed, in general, to contrast agents (CA), and methods and systems of using such agents for producing image contrast based on a magnetization transfer (MT) mechanism. The CA comprises a tetraazacyclododecane ligand having pendent arms R, R', R'' and R''' that are amides having a general formula: $-CR_1^1H-CO-NH-CH_2^2-R_2^2$. R_1^1 includes organic substituents and R_2^2 is not hydrogen. A paramagnetic metal ion (M) is coordinated to the ligand. The method, comprises subjecting a CA, in a sample, to a radio frequency pulse. The CA has pendent arms R, R', R'' and R''' comprising organic substituents and the ligand further includes a M and a water molecule. A signal is obtained by applying a radio frequency pulse at a resonance frequency of the water molecule. The magnetic resonance system, comprises a magnetic resonance apparatus and the CA, the agent containing a ligand having the above described general formula.

WO 02/043775 A3



— before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(88) Date of publication of the international search report:

18 December 2003

INTERNATIONAL SEARCH REPORT

Inter al Application No

PCT/US 01/46151

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K49/10 G01R33/28 A61P43/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K G01R

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, MEDLINE, BIOSIS, CHEM ABS Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ZHANG S ET AL: "A NOVEL PH-SENSITIVE MRI CONTRAST AGENT" ANGEWANDTE CHEMIE. INTERNATIONAL EDITION, VERLAG CHEMIE. WEINHEIM, DE, vol. 38, no. 21, 2 November 1999 (1999-11-02), pages 3192-3194, XP000864995 ISSN: 0570-0833 page 3192, column 2 page 3194, column 1	1-4,9
X	WO 00 47111 A (SHERRY A DEAN ;UNIV TEXAS (US); WU KUANGCONG (US); ZHANG SHANRONG) 17 August 2000 (2000-08-17) page 11, line 22-26; claims 14-20 page 10, line 5-8 figures 2-4,7,9,10	1-5,9
Y	page 16 ----- -/-	1-36

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

Z document member of the same patent family

Date of the actual completion of the international search

6 October 2003

Date of mailing of the international search report

14/10/2003

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Gonzalez Ramon, N

INTERNATIONAL SEARCH REPORT

Inte 1al Application No

PCT/US 01/46151

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93 12097 A (UNIV TEXAS) 24 June 1993 (1993-06-24) page 22; claims 21,22; figures 24,25,30; examples 6,7 ---	1-5,9
X	WO 91 03200 A (GEN HOSPITAL CORP) 21 March 1991 (1991-03-21) page 30 -page 31; claim 6 ---	1-6,9
Y	TANTTU J. I. ET AL: "Synergistic enhancement of MRI with Gd-DTPA and magnetization transfer" J. COMP. ASSIST. TOMOGR, vol. 16, no. 1, 1992, pages 19-24, XP008016911 abstract see discussion ---	1-36
A	JONES R A ET AL: "IMPROVING THE CONTRAST IN RAPID IMAGING SEQUENCES WITH PULSED MAGNETIZATION TRANSFER CONTRAST" JOURNAL OF MAGNETIC RESONANCE, ACADEMIC PRESS, ORLANDO, FL, US, vol. 97, no. 1, 1 March 1992 (1992-03-01), pages 171-176, XP000371667 ISSN: 1090-7807 page 171 ---	1-36
A	BALABAN R S ET AL: "MAGNETIZATION TRANSFER CONTRAST IN MAGNETIC RESONANCE IMAGING" MAGNETIC RESONANCE QUARTERLY, NEW YORK, NY, US, vol. 8, no. 2, 1992, pages 116-137, XP000982000 figure 4 ---	1-36
X,P	ZHANG S ET AL: "A novel europium(III)-based MRI contrast agent." JOURNAL OF THE AMERICAN CHEMICAL SOCIETY. UNITED STATES 21 FEB 2001, vol. 123, no. 7, 21 February 2001 (2001-02-21), pages 1517-1518, XP001151913 ISSN: 0002-7863 cited in the application abstract page 1518, column 1, paragraph 2 -column 2, paragraph 1; figure 2 ---	1-5, 7-12, 14-16,18

-/--

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 01/46151

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y,P	<p>ZHANG S ET AL: "DOTA-bis(amide)lanthanide complexes: NMR evidence for differences in water-molecule exchange rates for coordination isomers." CHEMISTRY (WEINHEIM AN DER BERGSTRASSE, GERMANY) GERMANY 5 JAN 2001, vol. 7, no. 1, 5 January 2001 (2001-01-05), pages 288-296, XP001151909 ISSN: 0947-6539 page 289, column 2; figure 1 page 294, column 2</p> <p>----</p>	1-36
T	<p>AIME SILVIO ET AL: "Paramagnetic Lanthanide(III) complexes as pH-sensitive chemical exchange saturation transfer (CEST) contrast agents for MRI applications." MAGNETIC RESONANCE IN MEDICINE, vol. 47, no. 4, April 2002 (2002-04), pages 639-648, XP001151918 April, 2002 ISSN: 0740-3194 see chart 1 page 641 page 640, column 1</p> <p>----</p>	1-6, 9-18,23
T	<p>ZHANG SHANRONG ET AL: "Unusually sharp dependence of water exchange rate versus lanthanide ionic radii for a series of tetraamide complexes." JOURNAL OF THE AMERICAN CHEMICAL SOCIETY. UNITED STATES 24 APR 2002, vol. 124, no. 16, 24 April 2002 (2002-04-24), pages 4226-4227, XP001151910 ISSN: 0002-7863 abstract see scheme 1 page 4227, column 2</p> <p>-----</p>	1-23

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 01/46151

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 10-23 are directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☒ Claims Nos.: partially 1-36
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA 210

Continuation of Box I.2

Claims Nos.: partially 1-36

Present claims 1-36 relate to an extremely large number of possible compounds/methods defined as formula as depicted in claim 1 wherein

a) R are "amides", R1 includes "organic substituents", R2 is "not hydrogen" (claims 1, 10, 14, 15, 22, 24); "does not have a proton exchangeable group" (claim 7);

b) R1 or R2 contain "alkyl group", "cycloalkyl group", "alkyloxy group", "alkyl ether", "polyol", "having 20 carbon atoms or less" (claims 8, 9).

Support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds/methods claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Moreover claims 2-4, 11, 29, 34-36 relate to compositions defined (inter alia) by reference to the following parameters: "exchange limiting regime" (claims 2, 11, 34), "lifetime of the exchange site" (claims 4, 36); "difference in frequency between the MR frequency of the exchanging sites and the MR frequency of bulk water" (claims 3, 29, 35). The use of these parameters in the present context is considered to lead to a lack of clarity within the meaning of Article 6 PCT. It is not fully possible to compare the parameters the applicant has chosen to employ with what is set out in the prior art. The lack of clarity is such as to render a meaningful complete search impossible.

Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the compounds/methods depicted in the examples in relation to their use as magnetic resonance contrast agents.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte _ if Application No

PCT/US 01/46151

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 0047111	A	17-08-2000	AU 3225900 A	29-08-2000
			WO 0047111 A1	17-08-2000
WO 9312097	A	24-06-1993	US 5316757 A	31-05-1994
			AU 2912492 A	19-07-1993
			CA 2125773 A1	24-06-1993
			CN 1073680 A , B	30-06-1993
			EP 0618910 A1	12-10-1994
			JP 7502994 T	30-03-1995
			MX 9207234 A1	01-06-1993
			NZ 244795 A	26-07-1995
			NZ 264440 A	26-07-1995
			PT 101123 A	28-02-1994
			WO 9312097 A1	24-06-1993
			US 5428155 A	27-06-1995
			ZA 9208338 A	04-05-1993
WO 9103200	A	21-03-1991	AT 173336 T	15-11-1998
			CA 2065290 A1	01-03-1991
			DE 69032761 D1	17-12-1998
			DE 69032761 T2	22-04-1999
			EP 0489869 A1	17-06-1992
			HK 1012707 A1	12-05-2000
			JP 5503072 T	27-05-1993
			WO 9103200 A1	21-03-1991
			US 5318771 A	07-06-1994
			US 5628982 A	13-05-1997
			US 5250285 A	05-10-1993

This Page is inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

☒ BLACK BORDERS

☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES

☐ FADED TEXT OR DRAWING

☒ BLURED OR ILLEGIBLE TEXT OR DRAWING

☐ SKEWED/SLANTED IMAGES

☐ COLORED OR BLACK AND WHITE PHOTOGRAPHS

☐ GRAY SCALE DOCUMENTS

☐ LINES OR MARKS ON ORIGINAL DOCUMENT

☐ REPERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY

☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images
problems checked, please do not report the
problems to the IFW Image Problem Mailbox**

This Page Blank (uspto)